ELECTROCHEMICAL DETECTION IN LIQUID CHROMATOGRAPHY: GRADIENT ELUTION, MICELLAR MOBILE PHASES, AND ENVIRONMENTAL APPLICATIONS

BY

MORTEZA GHOLI KHALEDI

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DEDICATION

To My Parents

and

My Love, Shahrzad

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To my companion and wife Shahrzad, her love, continuous support, infinite patience, and sacrifices have made my life so pleasant, have given me strength and inspiration when I needed them most, have contributed so much to my progress, and have made it so difficult for me to find proper words to thank her.

My best regards go to the friends and colleagues in Dr. Dorsey's group whose friendship made this time pass so easily.

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In his research group as a science student, I learned precious lessons, even more important than chemistry. I learned to challenge the problems rather than "escaping" from them or "hiding" them. I observed

the "magic" of hard work and determination. I learned how to use all the possible resources to sole the problems yet remain an independent researcher. His concern, patience, excellent advice, steady guidance, constant support, encouragement, and confidence building were instrumental in all these and my other achievements. I give my sincerest gratitude and appreciation to him, my friend and advisor, Dr. John Dorsey.

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LIST OF ABBREVIATIONS

EC	Electrochemical
LC	Liquid chromatography
MP	Mobile phase
RP	Reversed phase

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ELECTROCHEMICAL DETECTION IN LIQUID CHROMATOGRAPHY: GRADIENT ELUTION, MICELLAR MOBILE PHASES, AND ENVIRONMENTAL APPLICATIONS

By

Morteza Gholi Khaledi

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Oxidative electrochemical detection is used for liquid chromatographic analysis of polycyclic aromatic hydrocarbons (PAH). The amperometric response is shown to be dependent on the working potential, ionic strength, flow rate, pH, temperature, and pretreatment of the glassy carbon electrode surface. These experimental factors were optimized by choosing the maximum signal-to-noise ratio. Under optimized conditions traces of PAH's in the ng level can be detected with electrochemical detection. The analytical figures of merit, sensitivity, limit of detection, and linear range of 6 PAH's are reported and compared with UV absorption detection. The excellent selectivity of electrochemical detectors is demonstrated as only one of two or more overlapping compounds is detected by varying the applied potential.

The factors affecting baseline shifts in gradient elution are discussed. These parameters are applied potential, conductance, pH, impedance of the electrochemical cell, flow rate, viscosity, mobile phase impurity, temperature, and electrode sensitivity. The magnitude of residual current change is reported for water-methanol gradients at three different potentials. As expected the size of the baseline shift is far greater at high potentials where massive oxidation of the solvent occurs.

The extent of baseline shifts caused by micellar concentration gradients at different potentials, pH, and ionic strengths is studied. It is shown that the size of the residual current change can be greatly reduced, especially at high potentials, by balancing the pH and the ionic strength of the micellar solutions.

Cyclic voltammetry results for hydroquinone in micellar solutions are reported and compared to homogeneous aqueous solutions.

Adsorption isotherms of the surfactant are shown to demonstrate the fact that the composition of the stationary phase remains unchanged during a micelle concentration gradient above the critical micelle concentration. The strength of common micellar solutions is calculated and compared to methanol.

Finally a mixture of eight phenols is separated isocratically and with gradient elution, using a micellar mobile phase and amperometric detection. Separational selectivity of micellar mobile phase is discussed under isocratic and gradient elution. The compatibility of micellar gradient elution with electrochemical detector at high potentials is demonstrated.

CHAPTER 1

INTRODUCTION

Electrochemical Detection in Liquid Chromatography

It is becoming increasingly evident that for the analysis of complex mixtures a separation step is needed. Bonded phase reversed phase liquid chromatography has become the most widely used separation mode in high performance liquid chromatography (HPLC).[1] The combination of a non-polar stationary phase and an aqueous mobile phase offers the possibility of simultaneously separating a variety of compounds with different polarities.[1,2] However, a powerful separation technique can restore its meaning to quantitative and qualitative analysis only after it is followed by a suitable detection system. The search for an "ideal" detector for HPLC has attracted attention for years. An ideal detector is characterized as being sensitive; having a wide linear range; being stable and reproducible in signal in prolonged measurements; giving information to assist qualitative analysis; showing fast response; being independent of chromatograhic parameters such as flow rate, temperature, and mobile phase composition; having minimal contribution to band broadening; being easy to use and inexpensive; being nondestructive; and, finally, responding to all compounds and/or showing predictable selectivity. Regarding these characteristics it is sufficient to say that an ideal detector simply does not exist.[3-5] The enormous diversity of the complex mixtures to be analyzed makes the elusive idea of a universal detector for HPLC nothing but a false hope which creates more problems than it solves.[5-7] Among the HPLC detectors UV, fluorescence and electrochemical detectors have received widespread attention.

The combination of liquid chromatography with electrochemistry (LCEC) offers three distinctive advantages of selectivity, sensitivity and economy and has experienced rapid progress in the last decade.[6,8] The large volume of literature devoted to LCEC is an indication of ever-increasing interest in this powerful analytical technique. A total of 900 papers have been published on the subject, 650 of which are in just the past three years.[8] The most common EC detectors for HPLC are amperometric and coulometric detectors.[5] Conductometric,[9] high frequency impedance,[10] and potentiometric[11] detectors are also useful for continuous detection in flowing liquids[5] with the three latter being non-destructive.

Amperometric and coulometric detectors are sensitive, are selective, and can be applied to a broad range of inorganic and organic compounds. The applicability of electrochemical (EC) detectors to a given problem is a function of the electrochemical characteristics of the molecules of interest at a suitable electrode surface.[6] Electroactivity of the molecules is determined by their molecular structure while the thermodynamics and kinetics of the electrode process is a function of accessibility of various filled and unfilled molecular orbitals.[12] Many inorganic ions can be reduced or oxidized, while among organic compounds those with multiple bonds, reducible or oxidizable functional groups, aromatic systems, etc., are electroactive.[13] It should be noted that "electroactivity" is a function of the potential limit of the mobile phase and the electrode, i.e., on whether a specific oxidation or reduction potential falls within the accessible potential range. The major drawback of EC detectors is poor reproducibility which stems from a continuous change in electrode surface condition. This is due to various processes occurring at the surface such as adsorption, redox reactions, etc. Further disadvantages are the dependence of the signal and background current on flow rate, temperature, mobile phase composition. Electrochemical detectors have been applied mostly to the reversed phase mode where the mobile phase is polar. Their direct use in normal phase liquid chromatography (LC) with non-aqueous eluents of low dielectric constant has been thought to be unlikely;[6] however, in a recent report Gunashingham and Fleet[14] demonstrated the potential use of EC for the determination of phenols using hexane-ethanol solvent mixtures with tetraalkylammonium salts and ammonia as supporting electrolyte.

The working electrode materials commonly used are mercury, gold, platinum and carbon (glassy carbon, carbon paste, impregnated carbon, etc.). In general the factors to be considered in selection of a working electrode material are the accessible potential range, chemical and physical inertness to the mobile phase at a chosen applied potential, low noise and residual current, the degree of reproducibility of surface activity, and the kinetic parameters of the solute-electrode reactions.[13] In addition ruggedness and long term stability are also important parameters in LCEC.[6] Due to its wide cathodic potential range, mercury continues to be the proper electrode for electrochemical reductions; however, its great limitation remains a narrow anodic range in contrast to that of solid electrodes.[13,15] Unfortunately solid electrodes have a poor reproducibility and generally produce high residual currents and noise levels. This is mainly because of the surface inhomogeneity of the electrodes and their surface oxidation at positive potentials.[5] Among the solid electrodes, carbon paste has lower residual current and noise (in anodic range) and is easy to make and replace. Despite the slower electrode reaction on carbon paste compared to well polished glassy carbon or platinum, carbon paste exhibits very low detection limits, even slightly better than that of the former electrodes.[13,16,17] The major disadvantage of carbon paste[5,17,18] for use in HPLC is that it cannot be used with high concentrations of organic solvents (e.g., >20-30% methanol)[19] which tends to dissolve the impregnating liquid from the carbon. Because of the insufficient mechanical strength, carbon paste cannot be used at higher mobile phase flow rates. In addition owing to the reduction of adsorbed oxygen on and in the paste, the cathodic current is high.[18] The glassy carbon electrode is more versatile in terms of ruggedness and mechanical stability. It has a wide potential range (-0.80 V to +1.20 V vs SCE in aqueous buffer pH 4.5)[5] as well as excellent resistance to almost any solvent used in LC.[15,19]

The main disadvantage of glassy carbon is the loss of surface activity with use, which results in a decrease in sensitivity and poorer detection limits. The deactivation of the solid electrode surface is partly blamed on the adsorption of the analyte or redox reaction products, possibly forming polymer films.[20] The performance of glassy carbon is dependent on how "clean" the electrode surface is maintained during the course of the experiment. The electrode can be freed of adsorbed species by mechanical polishing of the surface;[21,23,24] chemical treatment of the surface with mineral acids, reductants and oxidants;[13,21] electrochemically by cyclic polarization of the electrode at suitable potentials;[21] or by a combination of these methods.[21-23,25] Polishing is reported to be the most effective method[5] and is usually followed by electrochemical pretreatment to reactivate the glassy carbon surface.[25] The procedure for cleaning and conditioning the electrode surface is especially tedious and time consuming for LCEC as it requires discontinuation of flow and dismantling the detector cell. Pulsed amperometric methods have been used for continuous cleaning of gold and platinum electrodes during the course of experiments.[26-28] By applying cleaning and measuring potential pulses alternately, the surface activity of the electrode can be maintained without any interruption in the measuring procedure. The cleaning potential value is different for various cases and must be measured for each specific case accordingly.[13] This method is probably ineffective for glassy carbon electrodes as the application of normal pulse and differential pulse waveforms results in chemical transformation of the electrode surface, producing much larger residual currents.[21] The technique, though effective in some cases,[26-28] is reported to fail frequently.[13] In summary despite the large number of pretreatment procedures developed by many workers,[21] there is still no general, easy and reliable method for the reactivation of the electrode surface.

In addition to the working electrode material, the detector cell design is another important factor that is to be considered in the application of EC detectors. To fulfill the basic HPLC detector requirement, the cell must have the smallest possible volume (few μL) and not interfere with the defined hydrodynamic condition.[5,13] From the electrochemical point of view the impedance between the electrodes should be minimal. The auxiliary and reference should then be placed as close as possible to the working electrode. Regarding the former requirements, the achievement of the latter is the most difficult problem in LCEC cell design. Most of the designs are based on the planar thin layer[5,18,6] and the wall jet cells.[13,18,24] A thin layer cell is shown in Figure 1 where the reference and auxiliary electrodes are placed downstream from the working electrode.[6] The cell volume is determined by the thickness of a gasket which is sandwiched between the two blocks. The working electrode surface is part of the channel wall of the lower (bottom) block.[6,29] The cell volume can be reduced by decreasing the spacer thickness which results in increasing the linear flow rate and thus better sensitivity; however, the rapid increase in noise with decreasing cell thickness is a limiting factor.[5,6] In addition the electric resistance between the electrodes increases with reduction in channel thickness. The major problem with the cell design, shown in Figure 1, is a large uncompensated solution resistance due to the large distance between the working electrode and auxiliary/reference electrodes. A typical cell resistance of 5 × 10^{+5}ohms is reported using an aqueous buffer whose resistance in a macroscopic conductometric cell is only about 300 ohms.[5] The interfacial electrode potential, i.e., the potential across the working electrode/solution interface E_i, is related to the applied potential E_w as follows:

$$E_i = E_w - E_{ref} - iR_u$$

where E_{ref} is the reference electrode potential and R_u is the uncompensated resistance of the solution; E_w and E_{ref} are constant;

therefore the E_i value is directly proportional to IR drop. As a result for the design where both auxiliary and reference electrodes are positioned downstream from the working electrode (Figure 1), a large current flow between the auxiliary and working electrode creates a severe IR drop problem and loss of control over working potential.[6,15] Background current and even the current generated due to peak elution can be the major contributor to the IR drop.[15,30] In addition due to the specific geometry in the thin layer cells, the current between the working and the downstream auxiliary electrode has to pass along the channel electrode surface. This generates IR drop along the surface and thus non-uniform polarization of the working electrode. At higher currents where the IR drop is larger, the potential difference between the upstream and downstream edges of the electrode is more pronounced resulting in a non-homogeneous rate of electron transfer along the face of the electrode.[6,15,31] This translates into a non-linear behavior of the detector when large samples are injected.[6,15] It also aggrevates the gradient induced baseline shift in EC detectors (see Chapter 4). The three electrode system compensates for a fraction of the solution resistance; this fraction is a function of the position of auxiliary and/or reference electrodes with respect to the working electrode; however, it has the advantage of not drawing current through the reference electrode which would result in the change of the reference electrode potential.[15] In addition the large resistance can be reduced by using high ionic strength media; however, concentrated salt solutions are not favorable in HPLC as they shorten the column lifetime. The best solution to the IR drop problem is to place the auxiliary electrode across from the working electrode which results in negligible uncompensated

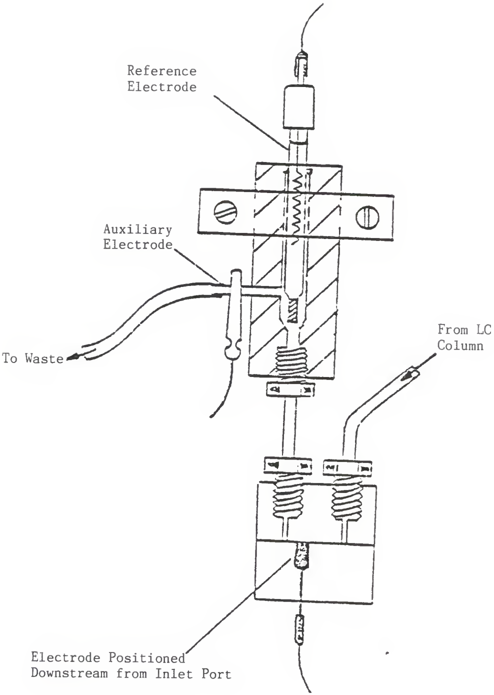


Figure 1. Thin-layer amperometric detector cell.

solution resistance even at ionic strengths as low as 10 mM.[6,15,30] This geometry also alleviates the non-uniform polarization of the electrode surface, as the charge passes perpendicular to the working electrode.[15] A more detailed treatment of the different thin layer cell designs is given by Kissinger et al.[15]

The need for a selective, sensitive and inexpensive analytical method for the determination of trace amounts of cathecholamines resulted in the development of LCEC.[6,8,32] Today the analysis of aromatic amines and phenolic compounds of biological, pharmaceutical and environmental importance comprises the bulk of LCEC applications.[5,6] Future research efforts will probably emphasize various on line/off line derivatization techniques in order to broaden the scope of LCEC applications.

Micellar Liquid Chromatography

Micellar solutions are composed of surfactants, also known as detergents or surface active agents. Surfactants are amphiphilic molecules composed of a hydrophobic tail joined to a hydrophilic (ionic or polar) head group. At low concentrations in aqueous solution, the surfactants mainly exist in a monomer form although dimers, trimers, etc., can exist.[33-36] At a critical concentration of surfactant known as the critical micelle concentration (CMC), the surfactant molecules assemble to form large molecular aggregates known as micelles. In aqueous media the polar head groups are directed toward and are in contact with aqueous solution while the hydrophobic or non-polar tail is oriented away from the polar media.[33-36] The hydrophobic repulsion of the non-polar tail with the aqueous environment, charge repulsion of ionic head groups, and the van der Waals attraction between the alkyl chains are believed to be the operating forces which result in micelle formation.[33] The shape of the micelles is reported to be symmetrical, even spherical, and its size is controlled by the number of surfactant molecules (typically 60 to 100) termed the aggregation number.[33,37] Above the CMC any change in total surfactant concentration only changes the concentration of micelles as the concentration of free surfactant remains almost constant and equal to CMC.

In liquid chromatography surfactants have been used extensively in order to shift the retention of overlapping ionogenic solutes through coulombic interactions. The technique is known as ion-pair chromatography, and the surfactant concentration was intentionally kept below CMC. The usefulness of micellar solutions serving as reversed phase mobile phases was first demonstrated by Armstrong and Henry.[38] A hydrophobic site for interaction with the solute is provided by micelles in the aqueous mobile phase and can be used in place of traditional organic modifiers. Armstrong and Nome[39] proposed a model based on solute partitioning between the stationary phase, micelles, and the bulk solvent (water) to explain the retention behavior of solutes in micellar liquid chromatography. Arunyanart and Cline Love[40] also developed a three phase equilibrium model relating capacity factor to micellar concentration in the mobile phase and derived equations to allow calculation of the equilibrium constant for the solute between the bulk aqueous phase and micellar aggregates. More significantly, if the equilibrium constant is available from independent methods, the equations can accurately predict the chromatographic capacity factor at zero or greater mobile phase micelle concentration.[40] This means that for the first time, spectroscopic measurements can accurately predict chromatographic retention, a goal not yet realized for hydro-organic mobile phases.[33] One of the major drawbacks in the early reports of micellar chromatography was a serious loss of efficiency when compared to hydro-organic mobile phases.[41] This problem has been overcome by Dorsey et al.[42] They demonstrated that efficiencies comparable to hydro-organic mobile phases can be achieved with the addititon of 3% propanol to the micellar mobile phase and column temperature of 40°C.

In micellar LC the concentration of micelles can be used to control the strength and selectivity of the mobile phase, similar to the role played by the organic modifier in hydro-organic mobile phases; however, the separation selectivity in micellar media is somewhat different from that of the hydro-organic, in a sense that the retention of solutes always decreases in the latter mobile phase by increasing the organic modifier concentration, while increasing the micelle concentration results in decrease,[39-42] increase, or no change[43] in retention. In addition due to the different rate of retention change (increase or decrease) for various solutes with the change in micelle concentration, even inversions in retention orders may be observed.[33,43] The different selectivity characteristics in micellar media stems from the variety of solute-micelle interaction (e.g., hydrophobic, electrostatic) which cannot be achieved with any combination of aqueous organic solution.[43]

Luminescence detection is reported to be improved in micellar media as for many solutes enhanced fluorescence[36,44] and, in some cases, room temperature liquid phosphorescence[44-46] is observed.

Electroanalytical techniques have often been applied to fundamental studies of micelles, but there is a paucity of information about electroanalysis in micellar solution. In an attempt to explore the application of micellar LC with amperometric detection, Hadjmohammadi[47] demonstrated that the analytical figures of merit (limits of detection, sensitivity, linearity, etc.) in micellar media are comparable to those of the hydro-organic mobile phases. The initial results show an excellent compatibility of micellar gradient elution with electrochemical detection using non-ionic surfactants.

Finally the noteworthy advantages of low cost and safety of micellar mobile phases compared to those of the hydro-organics should be considered.

CHAPTER 2

EXPERIMENTAL

Liquid Chromatography System

The system incorporated a Spectra Physics model 8700 ternary solvent delivery system (Spectra-Physics, Santa Clara, California) or an Altex (Berkeley, California) model 322 gradient liquid chromatograph with two model 100A dual reciprocating pumps. Either an Altex 210 or a Rheodyne (Cotati, California) injection valve with 5, 10, 20 μL loops and either an Altex ultrasphere octyl column (4.6 x 250 mm) or a Rainin microsorb octyl column (4.6 x 150 mm) both having 5 μm particle diameters were used. A dry hand-packed saturator precolumn was prepared using irregularly shaped silica gel of 25-40 μm diameter (Macherey-Nagel Company, West Germany) and was located before the injector. The mobile phase temperature was controlled by using water jackets for both the precolumn and the analytical column. Either a Neslab model 850 (NESLAB, Portsmouth, New Hampshire) or a HAAKE model D1 (Saddle Brook, New Jersey) bath circulator was used to thermostat the columns.

Detection System

The EC detector was an LC-4 amperometric controller which was connected to a 3-electrode thin layer detection cell (Figure 1; Bioanalytical Systems, West Lafayette, Indiana). The working electrode was glassy carbon and was polished to a mirror finish with 0.1 μm alumina (Fisher Scientific, Fair Lawn, New Jersey) and polishing paper (Buehler Company, Evanston, Illinois) and was pre-anodized at +1.50 v and precathodized at -1.50 v each for 5 min immediately before each experiment. The reference electrode was Ag/AgCl.

For the PAH study an Altex model 153 fixed wavelength 254 nm UV absorbance detector with an 8 L flow cell and time constant of 1.1 sec was placed before the EC detector.

A Waters (Waters Associates, Milford, Massachusetts) R401 refractive index detector was used for adsorption isotherm experiments and a Wescan (Wescan Instruments, Santa Clara, California) 213 conductivity detector was employed for gradient elution EC and adsorption isotherm experiments.

Cyclic Voltammetry System

A CV-1A cyclic voltammetry instrument and an electrochemical cell from Bio Analytical Systems were used. Glassy carbon and Ag/AgCl served as working and reference electrodes, respectively. An x-y recorder model RE0074 from PAR (Princeton, New Jersey) and a digital voltmeter were used as the recording system.

Reagents

Standard solutions of PAH's were prepared in the mobile phase solution (Table I). The mobile phase for PAH study was various ratios of HPLC grade acetonitrile (Fisher Scientific, New Jersey) and water, Using $NaClO_4$ as a supporting electrolyte. The deionized water was irradiated by UV light to remove trace organics. The surfactant was sodium dodecyl sulfate (SDS, Puriss grad) from Fluka (Fluka Chemical, Hauppauge, New York) and was used as received. Surfactant solutions were made in water:n-propanol (97:3) mixture and were filtered through a 0.45 μm nylon-66 membrane filter (Rainin Instruments, Woburn, Massachusetts). The phenols were dissolved in aqueous micellar solution.

For the micellar gradient-EC detector (compatability) experiments, the solvent H_2O:PrOH (97:3) was stored in a 6½ gal container to insure the homogeneity of impurities for all experiments.

CHAPTER 3

AMPEROMETRIC DETECTION FOR LIQUID CHROMATOGRAPHIC SEPARATION OF POLYAROMATIC HYDROCARBONS

Polynuclear aromatic compounds (PNA), including both polycyclic aromatic hydrocarbons (PAH) and heterocyclic compounds, are one of the most heavily studied classes of environmental pollutants. This widespread interest in their analysis stems from the demonstrated carcinogenic activity of many of these compounds. In fact the PNA's are the largest class of chemical carcinogens known today. These materials are produced in virtually all combustion processes, and analysis of these compounds is therefore of interest in air, water, sludges, and almost every conceivable type sample matrix. The PNA's are present in real samples as extremely complex mixtures, the compositions of which are mainly dependent on their sources, and can typically contain hundreds of components of widely ranging concentrations. The quantitation of these compounds, then, necessarily includes a separation step before measurement. The high resolving power of open tubular gas chromatography when dealing with complex PNA mixtures was demonstrated as early as 1964[48] and has been reviewed.[49] Gas chromatography-mass spectrometry (GC-MS) has also been used extensively for the quantitation of complex mixtures of PAH's.[50,51] However, the difficulties associated with distinguishing the mass spectra of isomeric PAH's from each other and inherent problems of GC such as the inability to handle high molecular weight or thermally unstable compounds are the major limitations of this technique.

Liquid chromatography has been receiving increasing emphasis in the separation of these complex samples. The ability to handle high molecular weight, thermally unstable compounds, and the possibility of using selective detection to determine overlapping or unresolved compounds makes HPLC ideal for analysis of these compounds. Detection has been performed predominantly by fluorescence emission and UV absorption, or both, with series detectors. Christiansen and May[52] have concluded that the most powerful system presently available is a 254 nm UV detector in series with a spectrofluorometer. This combination offers excellent limits of detection as well as some selectivity through the choice of excitation and emission wavelengths of spectrofluorometer. Recently an electrochemical detector was used for the determination of polycyclic aromatic amines.[53,54] Here the application of a new detection scheme, viz., oxidative electrochemical detection, which has heretofore not been applied to PAH's (Table 1) is discussed. The merits of this selective detector, such as sensitivity, limits of detection, and linear range, are compared with those of 254 nm UV absorption detection. Varying the applied potential gives the capability for selective detection of one of a pair of overlapping compounds.

The amperometric response is affected by numerous variables such as applied potential, temperature, flow rate and ionic strength of the mobile phase, size and composition of the working electrode, and detector cell design.[5,6,15] With the exception of the last three factors which were fixed by using a commercial EC detector, the others were optimized to achieve maximum signal-to-noise ratio. With a glassy carbon working electrode, other factors such as pretreatment of the electrode surface and pH also affect the detector response.

Table 1. PAH's used in this study

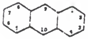
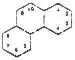
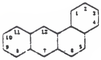
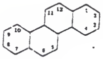
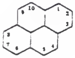
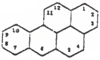
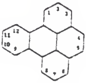
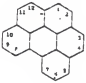
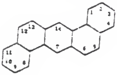
Name	Structure	EPA Priority Pollutant	Carcinogenic Activity[a]
Anthracene		+	0
9-Methylanthracene			0
2-Methylanthracene			0
9,10-Dimethylanthracene			0/+
Phenanthrene		+	0
Benz(a)anthracene		+	+
Chrysene		+	+
Pyrene		+	0

Table 1 - continued

Name	Structure	EPA Priority Pollutant	Carcino-ogenic Activity[a]
1-Methylpyrene			0
Benzo(a)pyrene		+	++
Benzo(e)pyrene			0/+
Benzo(ghi)perylene		+	+
Dibenz(a,h)anthracene		+	+

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[a] Reference 50: Non-carcinogenic - 0
Up to 33%, weakly carcinogenic - +
Above 33%, strongly carcinogenic - ++

Hydrodynamic Voltammogram

The optimum potential was determined from hydrodynamic voltammograms of each individual PAH, some of which are shown in Figures 2 and 3. The current was measured over a range of applied potentials (0.80 V - 1.40 V), and the potential at which the corresponding current reached the limiting current plateau was chosen as the working potential.[55] As these plots were measured on different days under varying conditions, the currents are not directly comparable, but the shapes of the curves are still valid. To maximize the analytical signal, the potential of 1.30 V (vs Ag/AgCl) which is on the limiting plateau for many of the compounds was used throughout the experiments. However, to demonstrate the selectivity of EC detectors for these compounds, other potentials from the rising part of the curves were chosen for the analysis of synthetic PAH mixtures.

Flow Rate

The current-flow rate relationship in thin layer electrochemical detectors has been studied previously.[56] Whether i (limiting current) is proportional to $U^{1/2}$ or to $U^{1/3}$ (flow velocity) for channel electrodes has been a matter of discussion for almost 30 yr.[57] However, to apply this concept in practice for chromatography, both have the same meaning; that is, current increases as flow rate increases. Here the electrochemical response of 7 PAH's was studied as a function of flow rate and is shown in Figure 4. The flow rate of 2 mL/min was chosen as optimum for all the compounds for further studies.

The exponent n obtained for seven PAH's (Table 2) shows the dependence of i on U^n. None of the values are exactly equal to 1/2or 1/3;

Figure 2. Hydrodynamic voltammogram for; △ 9,10-Dimethylanthracene (2.3 g), ■ 9-Methylanthracene (2 g), o Anthracene (2.1 g), □ 2-Methylanthracene (2.4 g); MP: (90:10) CH_3CN:H_2O, 0.1 M $NaClO_4$, F: 1 mL/min, T: ambient.

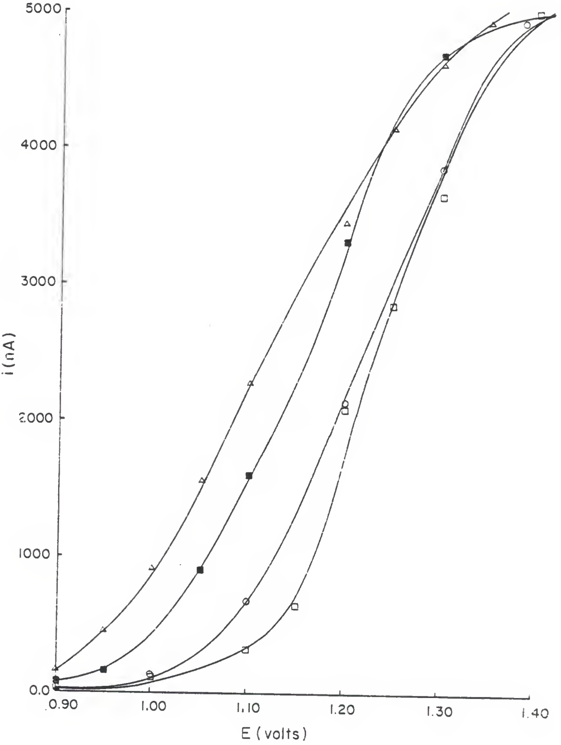

5000
4000
3000
2000
1000
0.0
i (nA)
0.90
1.00
1.10
1.20
1.30
1.40
E (volts)

Figure 3. Hydrodynamic voltammogram for: △ Benzo(a)pyrene (1.8 g), o 1-Methylpyrene (2.9 g), □ pyrene (2.2 g), ● Benzo(e)pyrene (2.0 g); other conditions as in Figure 2.

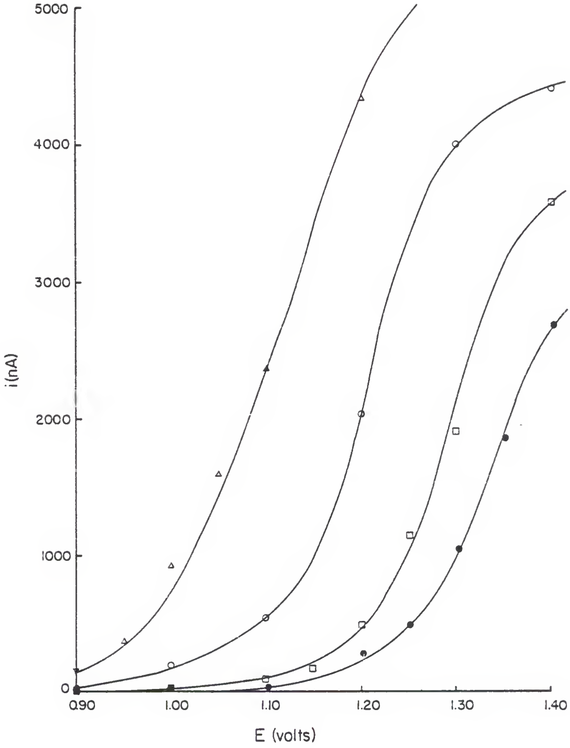

5000
4000
3000
2000
1000
0
i(nA)
0.90
1.00
1.10
1.20
1.30
1.40
E (volts)

Figure 4. Flow rate dependence of current: △ Anthracene (0.5 g), □ 9,10-Dimethylanthracene (1.9 g), o 1-Methylpyrene (0.12 g); E = 1.30 V vs Ag/AgCl, other conditions as in Figure 2.

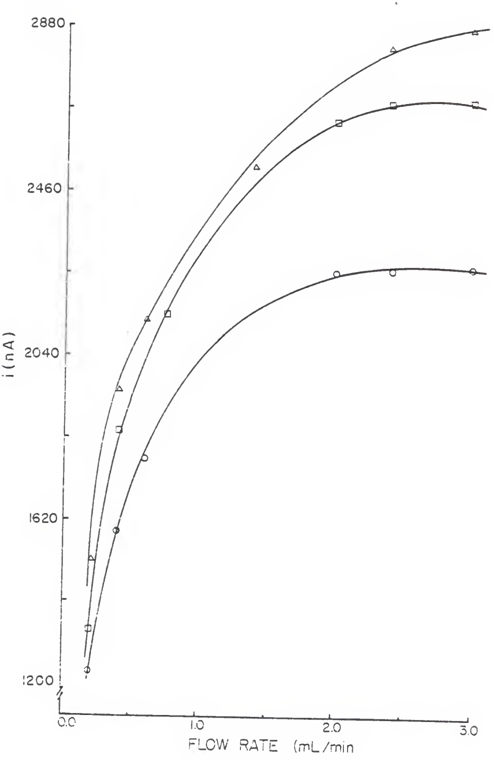

2880
2460
2040
1620
1200
i (nA)
0.0
1.0
2.0
3.0
FLOW RATE (mL/min

Table 2. Flow rate dependence of current: the exponent of flow rate for PAH's.

Compound	Exponent, n[a]
Anthracene	0.23
1-Methylpyrene	0.23
9-10,Dimethylanthracene	0.27
9-Methylanthracene	0.29
Benzo(a)pyrene	0.36
Pyrene	0.36

---

[a] $i \alpha U^n$
E = 1.30 V vs Ag/AgCl
Mobile phase: (90:10) $CH_3CN;H_2O$, 0.1 M $NaClO_4$
T: ambient

however, it can be concluded that for four of the PAH's, 9,10-dimethylanthracene, 9-methylanthracene, benzo(a)pyrene and pyrene, the limiting current dependence on flow rate falls in the $U^{1/3}$ range.

pH

The response of glassy carbon electrodes can be affected by pH variation,[58] manifesting itself in at least an apparent potential shift of hydrodynamic voltammograms. The signal-to-noise ratio at different pH's was measured for anthracene and benz(a)anthracene at a potential of 1.3 V and was found to be essentially constant. However, the pH of the mobile phase was adjusted to 7.5 for the rest of the study to prevent any possible changes in the EC response when the detector was operated at potentials lower than the limiting plateau.

Pretreatment of Glassy Carbon

The effect of electrochemical pretreatment on the response of glassy carbon, in terms of half wave potentials, has been reported.[25,59] The influence of both polishing and electrochemical preconditioning of the electrode surface on signal-to-noise ratio and reproducibility for anthracene and benz(a)anthracene is shown in Table 3. The results from no pretreatment, where the electrode was neither polished nor pretreated electrochemically, were similar to those where the electrode was either only electrochemically pretreated or only polished. However, poor reproducibility was observed for the first two cases where the electrode was not polished. Better signal-to-noise ratio and reproducibility were achieved when the electrode surface was polished mechanically and preconditioned electrochemically. In order to achieve consistent results, it is crucial to reproduce the surface conditions of the electrode. An operating potential of 1.30 V is large enough to oxidize the impurities present from the supporting electrolyte and water of the mobile phase. For this reason high operating potentials for LCEC have never been popular. The formation of an oxide layer on the carbon surface has been reported, once the anodization is accomplished at potentials where oxygen evolution is possible.[25,60] Engstrom reported that the oxide layer strongly inhibits the oxidation of ferrocyanide and hydrazine, but has no effect on hydroquinone oxidation.[25] The application of 1.30 V operating potential after electrode pretreatment may result in the formation of this oxide layer. If the layer inhibits the PAH's oxidation, the whole process of pretreatment is simply invalidated. However, such behavior was not observed: the pretreatment procedure always yielded better results. This was also verified by cyclic voltammetry. The voltammograms of both blanks and anthracene solute in mobile phase solution were exactly the same for freshly pretreated electrodes and those where the electrode was held at 1.30 V for 30 min after the pretreatment procedure. The polishing, preanodization and precathodization duration and techniques were carefully reproduced. A reciprocating motion was used for polishing, in contrast to a rotary motion which could produce large background currents due to turbulent flow on a microscale at the electrode surface.[61] A reproducible background current of 450 nA for the surface area of 0.07 cm^2 was observed. This high background current is undoubtedly due to the high operating potential. The peak-to-peak noise measurements were made for 20 min prior to the first injection when the electrode surface was freshly polished and pretreated. The entire pretreatment method was chosen from the literature.[25] A detailed study of the pretreatment procedure could give better results for PAH's, as its

Table 3. Effect of pretreatment on glassy carbon response.

Polished	Electrochemically Pretreated	Anthracene SNR	Benz(a)anthracene SNR
-	-	44	10
-	+	72	18
+	-	64	15
+	+	166	60

MP: (80:20) CH_3CN/H_2O, 0.01 M $NaClO_4$, pH: 7.50, column 150 4.6 mm, Rainin C-18 Microsorb; E = 1.30 V vs Ag/AgCl, F = 2 mL/min, T = 30°C, 0.2 μg injected.

effectiveness is reported to be dependent on the electrochemical species.[25]

Temperature

The rate of diffusion of molecules to the electrode surface and the rate of oxidation of molecules reaching the surface are both dependent on temperature. A temperature fluctuation of one degree (C) can cause from 1.5% to 9% change in the amperometric response.[62] In this experiment only the column temperature was controlled, and the EC detector cell was held at ambient temperature. Heat transfer between mobile phase, cell body, and the environment could cause some error. However, increasing the flow rate through the system and operating the electrode potential on the limiting current plateau are reported to minimize the temperature effect.[62] The amperometric response increases with the temperature because the limiting current for a thin layer electrode is proportional to the two-thirds power of the diffusion coefficient, and the rate of oxidation of molecules is also a function of temperature.[62,63] However, both peak-to-peak noise and background current also increased dramatically with increasing temperature. As a result a maximum signal-to-noise ratio (SNR) was observed at 23°C, and this was chosen as the optimum temperature for the analytical figures of merit study (Table 4). This occurrence of maxima is also reported by Miner who studied the effect of temperature on electrochemical response of other compounds.[62] While small changes in temperature have only limited effects on chromatographic resolution, changes of only a few degrees have been shown to cause elution order reversal for certain PAH pairs.[64] Column thermostating is then mandatory for reproducible separation of these compounds.

Table 4. Effect of column temperature on SNR.[a]

T (°C)	Anthracene SNR	Benz(a)anthracene SNR
15	157	37
23	405	105
30	157	46
37	99	24

---

[a] Conditions same as in Table 3.

Concentration of Supporting Electrolyte

A sodium perchlorate concentration of 0.025 M was used for the measurement of the analytical figures of merit. Using a higher concentration of supporting electrolyte will likely yield a greater linear dynamic range as it reduces the uncompensated resistance of the solution.[6] On the other hand lower salt concentration may be advantageous in terms of extended column lifetimes. No SNR dependence on background electrolyte concentration was found.

The analytical figures of merit of 6 PAH's for both UV and EC detectors are reported in Table 5. The limit of detection (LOD) defined as 3 times the standard deviation of blank/sensitivity, is in the sub-nanogram range for both EC and UV detectors. The LOD in chromatography is an ambiguous number, being dependent on both the detector and chromatographic variables such as capacity factor, void volume, column efficiency, and injection volume.[65] By operating the two detectors in series these variables were held constant, and a comparison of the detectors is valid. Care must be exercised, however, in comparing these values with others obtained under different chromatographic conditions. As can be seen the UV detector's LOD is better than the EC detector by almost an order of magnitude for both anthracene and 9,10-dimethylanthracene. This is because the wavelength of the detector, 254 nm, happened to be similar to λ_{max} for anthracene (λ_{max} = 253 nm) and 9,10-dimethylanthracene (260 nm). The wavelengths of maximum absorption for PAH's under study are also listed in Table 5.

Table 5. Analytical figures of merit for PAH's.

Compound	LOD				Sensitivity	
	EC		UV			
(MW)	ng	pmol	ng	pmol	nA/ng	AU 10^{-4}
Anthracene (178)	0.45	2.5	0.03	0.2	2.0	30.7
Pyrene (202)	0.82	4.1	0.41	2.0	1.1	2.2
Benzo(a)pyrene (252)	0.34	1.3	0.23	0.9	2.7	4.0
9,10-Dimethylanthracene (296)	0.31	1.5	0.070	0.3	2.9	12.8
1-Methylpyrene (216)	9.42	1.9	0.56	2.6	2.2	1.6
Benzo(ghi)perylene	0.39	1.4	0.53	1.9	2.3	1.7

Exp. condition = MP (80:20) CH_3CN, 0.025 M $NaClO_4$, F = 2 mL/min, T = 23°C, pH: 7.50, E = 1.30 V vs Ag/AgCl, glassy carbon pretreated.

a The concentration after which the linearity deviates by 5% or more.

Table 5 - extended

Upper Concentration of LDR[a]		Slope (log/log) of Calibration Curve		
EC (ng)	UV (ng)	EC	UV	λ_{max} (nm)
240	240	1.02	0.95	253
400	2,800	1.03	0.96	334
210	2,100	1.00	0.99	331
250	750	1.06	0.97	260
260	2,600	1.04	0.97	342
200	2,000	0.92	1.00	331

Selective Detection

The ideal chromatographic analysis would successfully resolve all compounds in a complex sample. This is quite likely an impossible goal for most real PNA matrices. Certainly a satisfactory and very achievable goal would be to have methodologies for the analysis of any desired component or components of a mixture. The need for complete chromatographic resolution is obviated if only one of two overlapping compounds is detected. Electrochemical detection provides another source of selectivity for these compounds, as will be shown.

Two mixtures of standard solutions, each containing 6 PAH's, were prepared. The chromatograms of mix A (Figures 5-8) and mix B (Figures 9 and 10) are shown. As can be seen from the UV detector's response at 81% CH_3CN, phenanthrene overlaps with anthracene (Peak 1, 2) while 9,10-dimethylanthracene, benz(a)anthracene and chrysene (Peaks 3-5) are virtually unresolved (Figure 5). The EC detector at E = 1.05 V detected only 3 of the PAH's in the mixtures (Figure 6). Changing to a weaker mobile phase composition of 67.5% CH_3CN results in the separation of phenanthrene from anthracene, and 9,10-dimethylanthracene from the still unresolved peaks of benz(a)anthracene and chrysene (Figure 7). Using the same mobile phase composition and applying a potential of 1.25 V results in selective detection of benz(a)anthracene in the presence of chrysene (Figure 8). The UV response for mix B, at 70% CH_3CN, is shown in Figure 9, with the pyrene and 9-methylanthracene peaks overlapped. Benzo(a)pyrene, 9-methylanthracene and 2-methylanthracene were selectively detected by EC detection at 0.95 V (Figure 10).

In real samples one can find a variety of PAH's with a wide range of concentrations. As a result, the familiar problem of simultaneous detection of major, minor and trace concentrations should be addressed. Examples of such problems are illustrated in the UV detector's chromatograms (Figures 5 and 9). For instance comparison of the UV response of benz(a)anthracene (16 ppm) and pyrene (22.4 ppm) with that of the 2-methylanthracene (10.4 ppm) (Figure 9) shows that although the concentrations of the former compounds are larger (1.5 to 2 times) than the latter one, their peak heights are almost 100 and 5 times smaller than 2-methylanthracene's. As another example in mix A, anthracene's response (9.6 ppm) is 15 times greater than that of the 1-methylpyrene (10.4 ppm), despite the equal concentrations. This problem is more pronounced if trace or minor concentrations of large PAH compounds with low molar absorptivities at 254 nm are to be analyzed simultaneously with major concentrations of smaller PAH's with high 254 nm molar absorptivities. However, as shown in Figures 8 and 10, this is not the case for EC detection where benzo(a)pyrene's and 1-methylpyrene's response are similar to those of the 9-methylanthracene and anthracene at 0.95 V and 1.25 V, respectively. Table 5 gives a more complete comparison of the sensitivities of UV and EC detection for PAH's.

Figure 5. Mix A: UV detector (254 nm) peaks (1) Phenanthrene (1.3 n mol), (2) Anthracece (1.1 n mol), (3) 9,10-Dimethylanthracene (0.97 n mol), (4) Chrysene (0.98 n mol), (5) Benz(a)anthracene (1.1 n mol), (6) 1-Methylpyrene (0.96 n mol); MP: (81;19) $CH_3CN:H_2O$, F: 1 mL/min, column: Rainin 150 × 4.6 mm, microsorb C-18, 5 μm, T = 27°C.

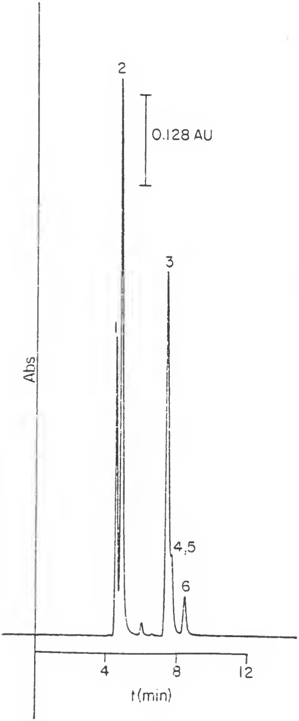

2
0.128 AU
3
1
Abs
4,5
6
4
8
12
t(min)

Figure 6. Mix A: EC detector, E = 1.05 V vs Ag/AgCl, other conditions as in Figure 5.

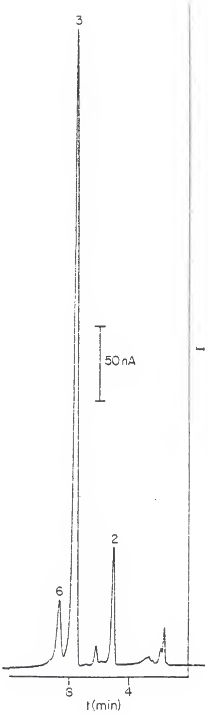

3
2
6
1
50 nA
3
4
t(min)

Figure 7. Mix A, MP: (67.5:32.5) $CH_3CN:H_2O$, other conditions as in Figure 5.

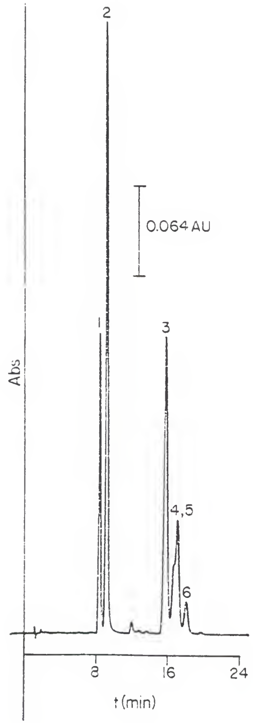

2
0.064 AU
1
3
Abs
4,5
6
8
16
24
t (min)

Figure 8. Mix A, MP: EC detector, E = 1.25 V vs Ag/AgCl, other conditions as in Figure 7.

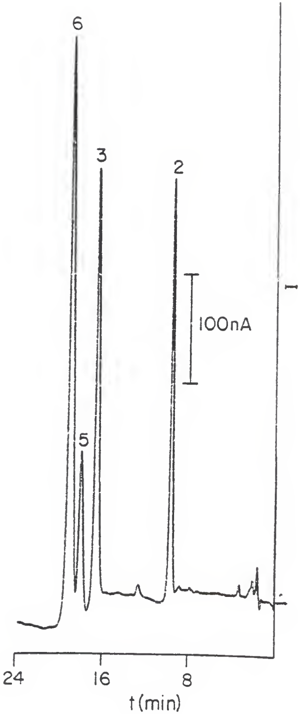

6
3
2
5
100nA
24
16
8
t (min)

Figure 9. Mix B: UV detector, peaks (1) 9-Methylanthracene (1.3 nmol), (2) Pyrene (2.2 nmol), (3) 2-Methylanthracene (1.1 nmol), (4) Benzo(e)pyrene (0.95 nmol), (5) Benzo(a)pyrene (0.7 nmol), (6) Dibenz(a,h)anthracene (1.2 nmol); MP: (70:30) $CH_3CN:H_2O$, other conditions as in Figure 5.

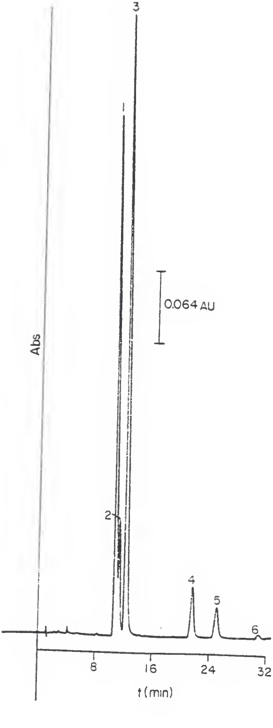
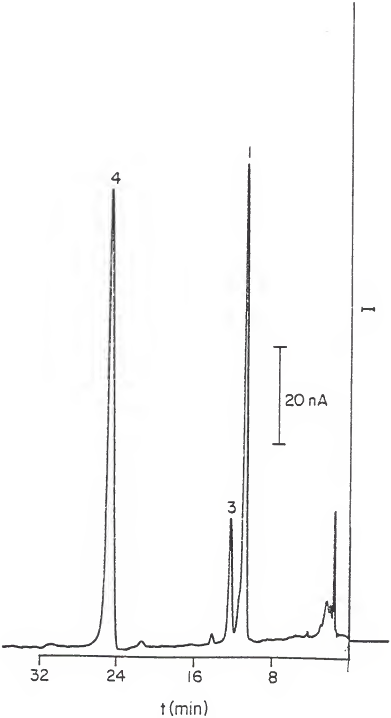

3
1
2
4
5
6
0.064 AU
Abs
8
16
24
32
t (min)

Figure 10. Mix B: EC detector, E = 0.95 V vs Ag/AgCl, other conditions as in Figure 9.


4
1
3
20 nA
32
24
16
8
t (min)

CHAPTER 4

GRADIENT ELUTION LIQUID CHROMATOGRAPHY WITH EC DETECTORS

The Question of Compatibility

To many workers one of the major drawbacks of EC detectors is their limited applicability to gradient elution.[13,17] It is not uncommon to read in LCEC literature, EC detectors are often incompatible with gradient elution.[13] Some even believed that gradient elution cannot be used with EC detectors.[17,66] Their logic is based on the fact that background current is dependent on electrolyte composition, so any change in mobile phase composition would shift the baseline. Recently, however, some authors have reported, Contrary to previous reports, the compatibility of LCEC with gradient elution is excellent.[67] This is based on the successful experience of some workers in using gradient elution with EC detectors.[67-69] Different experimental conditions have caused this contradiction. Undoubtedly in order to answer the problem, one should study the parameters involved, the importance of each in contributing to the shift, and whether it is possible to decrease the gradient induced baseline shift to an acceptable value by adjusting the contributing factors. Surprisingly, however, despite the large number of publications on LCEC, no one has addressed the issue in detail. That is why in one case EC detectors show excellent compatibility while in others they cannot be used with gradient elution. It should be mentioned that some workers have briefly mentioned the parameters involved; however, their efforts are far from a comprehensive study of the subject.[69,70]

A study of components of residual current under isocratic condition and the factors affecting it should help us to achieve a better understanding of those parameters contributing to gradient induced baseline shift.

Residual Current in Amperometric Detection

The application of potential to the working electrode generates a large transient current, known as charging current, which decays rapidly. The steady state background current is composed of two components. Firstly, the residual current results from electrochemical processes associated with the electrode surface. For glassy carbon electrodes these processes extend from the oxidation of the functional groups on the electrode surface and formation of oxide layer(s) whenever oxygen evolution occurs to the dissolution of adsorbed species and oxide layer, etc., depending on the past history of the solid electrode.[23,25] The second component is a result of electrolysis (oxidation in this work) of traces of electroactive impurities in the solution and/or oxidation of the solvent itself. Thus the magnitude of the residual current is dependent on the surface condition of the solid electrode and the rate of impurities and solvent oxidation. The extent to which the impurities or MP undergo oxidation is a function of bulk MP properties such as conductivity, pH, etc., along with the magnitude of the working potential.

In general the following factors are related to residual current: 1) applied potential, 2) EC cell design, 3) ionic strength of eluent,

4) pH, 5) viscosity, 6) flow rate 7) temperature, 8) mobile phase purity, and 9) electrode sensitivity.

Potential

The applied potential can affect the background current in three ways. Firstly, the extent of electrolysis of mobile phase impurities is dependent on the potential. Secondly, it determines whether the solvent (e.g., water) is being oxidized or not depending on how close its value is to that of the solvent decomposition potential. Finally, the condition of the electrode surface is a function of working potential. In summary, by operating at "high" potential (e.g., >1.20 V vs Ag/AgCl), more impurities undergo oxidation, solvent decomposition is more likely to happen, and the electrode surface is largely affected, overall generating a larger background current.

Cell Design

The EC cell design, although it does not directly affect the magnitude of the residual current, is an important consideration in that it determines the impedance between the electrodes. In other words, the ohmic potential drop which directly affects the applied potential varies by changing the position of the electrodes. In non-aqueous solvents the problem of IR drop is severe because of poor conductivity. The three electrode system is used to suppress the effect of IR drop, and the reference and auxiliary electrode should be placed close to the working electrode. As was mentioned in previous chapters, the resistance between the electrodes in the cell used in this study is quite large. This is due to the fact that the reference electrode is positioned away from the working electrode. The ohmic drop problem in thin layer cells is somewhat different from the usual electrochemical cell.[15] This is due to the channel electrode geometry and the fact that the reference and auxiliary electrodes are located downstream with respect to the working electrode. As a result the current between the working and auxiliary electrodes passes along the surface of the working electrode, thus creating a significant amount of IR drop along the surface of the channel electrode rather than perpendicular to it. Therefore the potential across the surface of the working electrode is non-uniform, and the downstream edge of the electrode has the closest potential to the value controlled by the potentiostat.[15] Consequently the IR drop problem is even more severe when the background current is large, e.g., at high applied potentials. The non-uniform polarizatiton of the electrode limits the linear response of the detector and can be a major contributor to baseline shift in gradient elution.

Ionic Strength

The importance of the ionic strength of the eluent is because it influences the ohmic resistance of the medium. Conductivity does not directly affect the background current, but when coupled with the cell geometry factor (i.e., IR drop in EC cell), it can play an important role. In the cell designs with low impedance between the electrodes (e.g., placing the auxiliary electrode across from the working electrode), a small uncompensated resistance can be achieved even with solutions of low conductivity;[6] otherwise a change in ionic strength can have a profound effect on the IR drop and thus the residual current.

pH

One of the major constituents in RP eluents is water. The oxidation potential of water is a function of pH, as H^+ is one of the products of water oxidation: $2H_2O \rightarrow O_2 + 4H^+ + 4e^-$. Thus $E = 1.23 - 0.059$ pH, considering the pressure of O_2 as 1 atm and NHE as reference electrode. In general water can be oxidized more easily as the pH increases thus producing larger background currents. In addition, H^+ is also involved in glassy carbon surface redox reactions.[71] Thus pH can influence the surface condition of the electrode[71] and probably the magnitude of residual current.

Viscosity

The effect of viscosity and flow rate on background current can be understood in terms of transport rate of electroactive impurities to the electrode surface. This mass transport in flowing systems is by diffusion and convection. The resulting current from electrolysis of impurities is then a function of flow rate, viscosity and diffusion coefficient, acknowledging the fact that diffusion coefficients are also a function of viscosity. Therefore more residual current is observed at higher flow rates with less viscous media and large diffusion coefficients. In addition the frictional heat induced by eluent traversing the column is a function of viscosity. Recalling the background current dependence on the temperature, one should conclude that viscosity can affect the residual current as both of these are related to temperature.

Temperature

The temperature effect on EC signals is discussed in previous chapters. The temperature dependence of background current is similar to that of the analyte because temperature influences the rate of diffusion of impurity molecules to the electrode surface as well as the rate of electron transfer. Hence the background current increases with temperature.

Mobile Phase Purity

The role of mobile phase purity is clear. Electrochemically pure solvents produce low residual current.

Electrode Sensitivity

The electrode sensitivity is a function of the "cleanness" of the electrode surface which influences the size of background current and reproducibility of results. It is related to pretreatment frequency and procedure. One of the common methods (which was also used in this work) consists of polishing the surface followed by alternate polarization of the electrode. A well polished glassy carbon surface is reported to be virtually free of functional groups;[21] however, carbonyl groups and even carboxyl groups can be formed as the electrode is immersed in a solution containing strong oxidizing agents and/or anodic potential is applied.[21] Laser and Ariel[60] confirmed the existence of surface groups. They concluded that the anodic polarization results in the surface group formation such as carbonyl groups that subsequently can be reduced to hydroxyl group at more negative electrode potentials. Also the formation of quinone/hydroquinone cannot be ruled out.[21,60] Dieker et al.[72] also supported these results and demonstrated that the large residual currents observed at glassy carbon electrodes are largely due to redox reactions of the electrode surface rather than electroactive impurities or charging the electrical double layer. A "clean" electrode surface results in smaller background currents and more reproducible results.

Parameters Causing Baseline Shift

It is quite clear that those factors which affect the residual current are also the cause of the shift. Variation of these parameters during gradient elution alters the extent of electrolysis of the mobile phase and impurities, thus resulting in a residual current change. Also any change in the electrode surface condition due to changes in mobile phase composition contributes to the baseline shift. In addition it is known that the change in double layer caused by solvent composition variation induces charging current flow (at least transiently).[73] Therefore the possibility of charging current contribution to baseline shift cannot be excluded. It should be noted that these factors are not independent of each other, and the extent to which they are involved in baseline disturbances could be enhanced or reduced by the existence or the lack of one or more others. For instance, working potential is fixed in amperometric detector, and there is not any direct relation between residual current, applied potential, and solvent composition. However, the gradient induced baseline shift is greater at higher potentials. Obviously EC cell design is another fixed parameter, but it affects the background current to a large extent. Both of the mentioned parameters are closely related and once joined by the changes in dielectric properties of the media due to gradient elution, they play crucial roles in the magnitude of the baseline shift. The key factor among the three is the EC cell design or the impedance between the electrodes which determines the extent of IR drop along the electrode surface and between the electrodes. Considering high impedance cells, such as the one used in this work, the major contributor to potential drop is the background current itself, whose magnitude is largely dependent on operating potential. In gradient elution the IR drop changes as a result of variations in conductivity and so the uncompensated resistance of the media. Thus the applied potential along the electrode surface is continuously changing during the course of the gradient, and therefore the extent of electrolysis of impurities and the mobile phase is varied. The surface condition of the electrode as well as the double layer may be altered. Consequently the residual current changes and the baseline shifts. Assuming other factors to be constant the magnitude of the shift is dependent on the applied potential, the value of cell impedance, and the extent of conductivity change, i.e., gradient range. For instance, using a high impedance cell but operating at low potentials (less than solvent decomposition potential) where the residual current and the IR drop are "small," the changes in conductivity (other factors constant) would vary the potential. However, the resulting potential change is not "large" enough to change the impurity electrolysis or the electrode's surface condition to a great extent, thus the resultant baseline shift is minimal. Using a low impedance cell so that the IR drop is negligible even at low ionic strengths minimizes the baseline shift dependence on the potential even for large variations in mobile phase conductivity. Finally it is possible to decrease the background current change to a "tolerable" value even with a high impedance cell and high working potential providing the conductivity of the mobile phase is held constant. The result of the uncompensated resistance of the unchanging solution is a constant IR drop. Although the IR drop still exists along the electrode surface and the electrode is yet polarized nonuniformly, that does not cause the baseline to shift. It should be emphasized once again that in the study of the effect of potential, cell design, and conductivity, other factors were assumed constant for the simplicity of the discussion. This assumption might not be totally correct for all cases.

Viscosity variation in the gradient process affects the diffusion coefficients of the electroactive species and thus their transfer rate to the electrode surface. It can also induce frictional heat in the column causing a temperature change.[69] As mentioned earlier background current increases with temperature. Therefore it is important to thermostat the entire chromatographic system (e.g., column, EC cell) to ensure the minimization of temperature induced baseline shifts. Another parameter which should be watched closely is the pH. The rate of oxidation of water or other species whose oxidation potential is a function of pH is altered by the pH variation. The baseline shift can be influenced greatly by pH gradients, especially at potentials high enough to cause solvent decomposition. Variation in concentration of electroactive impurities obviously does cause baseline shift. Using electrochemically pure reagents minimizes the problem. Flow rate is usually held constant during a concentration composition gradient, and flow fluctuations are usually too small to be considered seriously. However, a flow gradient creates residual current changes due to variation in transfer rate to the surface of the electrodes. Changes in electrode surface condition could greatly affect the background current variation. This is likely to occur during a gradient run since the solvent composition at the interface of the surface is changing. This factor is more pronounced at higher potentials where one of the mobile phase constituents is being oxidized. For example, the oxygen evolved from oxidation of water is sorbed on the glassy carbon surface forming an oxide layer or layers. Replacing the water content with an organic modifier during a gradient run alters the characteristics of the electrode surface at the solution interface; thus the size of the electrode's contribution to residual current may vary. The "cleaner" the electrode, the lower the background current and shift will be.

The Role of Solvent in Gradient Induced Baseline Shift

The change of solvent type such as water to methanol as well as continuous potential change along the electrode surface during a gradient run does change the structure and capacitance of the double layer at a solid electrode. Whether in amperometric mode these variations contribute significantly to baseline shift in the form of charging current is not known as the double layer structure at solid electrodes is not yet fully understood. Assuming the change in double layer structure results in charging current generation, the problem can then be discussed in two different perspectives. On one hand the charging current is a transient signal being generated for a few msec. Its lifetime is much shorter than the gradient time period of several minutes. Therefore it dies out during the course of the gradient and would not affect the background current. On the other hand one can argue that as a result of the continuous change in solvent composition, charging current might be generated continuously. Comparing it to other factors, the contribution of charging current to the residual current shift, if any, is probably not very significant. As will be seen later, the baseline disturbance is drastically reduced once the other parameters are controlled.

The extent of baseline shift was studied for hydro-organic and micellar gradients under different conditions. The flow rate was held constant, and the column and precolumn were thermostated. The electrode was pretreated mechanically and electrochemically on a periodic basis, often before each experiment, to minimize any baseline shift due to electrode fouling. The water component of the mobile phase was deionized and irradiated with uv light to minimize impurities. Other reagents in the mobile phase were used as received. It is believed that major contributors to the baseline change are potential, EC cell design, conductance, and the pH of the media. As these factors are closely related, it was concluded that monitoring the conductance and pH of the solution was of critical importance. A conductivity detector was then used in series with the amperometric detector.

Hydro-organic Gradient

Figure 11 shows the specific conductivity of a 0.005 M $NaClO_4$ water-methanol mixture as a function of methanol percentage. Figures 12 a-c are the amperometric detector traces for the gradient of water to methanol solution at three different potentials of 0.80 V, 1.0 V, and 1.20 V. The degree of gradient compatability was judged in terms of the change in residual current. Because of the dependence of background current on the condition of the glassy carbon surface, electrode fouling can have dramatic effects on the result. Therefore the value of the residual current and the amount of change can differ more than 100% for the same experimental conditions at different times. A pretreated electrode usually shows good reproducibility. In addition to repetitive runs, similar or exact results, in terms of the size and the trend of residual current change obtained for both forward and reverse gradients (i.e., methanol to water), were taken as a measure of reproducibility.

Considering the different sensitivities used for 0.80 V and 1.0 V compared to 1.20 V, one should realize that the gradient induced

Figure 11. Specific conductance vs percentage of CH_3OH in a H_2O-CH_3OH mixture (0.05 M $NaClO_4$).

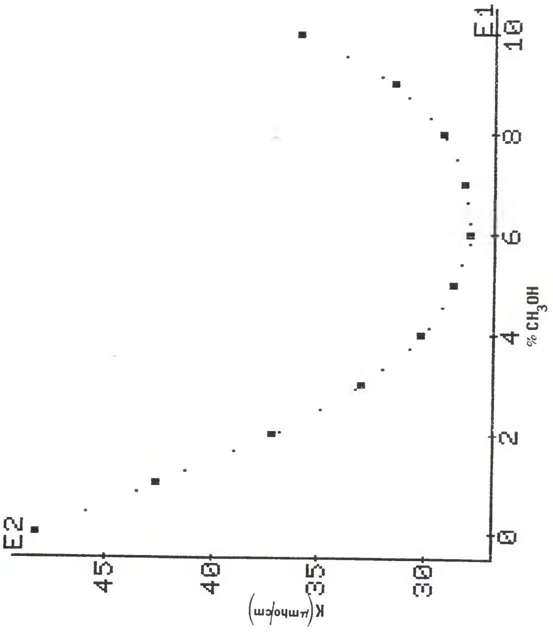

E2
45
40
35
30
K(μmho/cm)
0
2
4
6
8
10
E1
% CH_3OH

Figure 12. Hydro-organic gradient induced baseline shift.

(a) E = 1.20 V vs Ag/AgCl

(b) E = 1.00 V vs Ag/AgCl

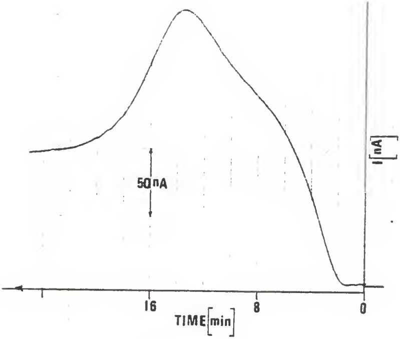
(c) E = 0.80 V vs Ag/AgCl

Solvent A: 0.05 M in H_2O

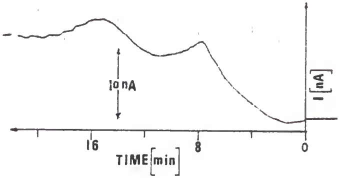
Solvent B: 0.05 M in CH_3OH

Gradient Program: A to B in 5 min

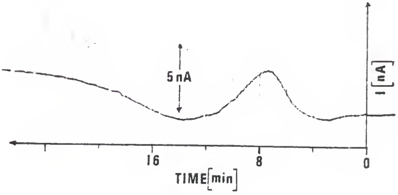
F: 1 mL/min; ambient temperature; glassy carbon electrode a


50 nA
I [nA]
16
8
0
TIME [min]

b


10 nA
I [nA]
16
8
0
TIME [min]

c


5 nA
I [nA]
16
8
0
TIME [min]

background current change for 1.20 V is much higher than 0.80 V and 1.0 V even though the changesin mobile properties such as conductance, viscosity, pH, etc., are equal for all the three potentials. Although the effect of potential on residual current magnitude is quite clear, the dependence of baseline shift on potential cannot be explained unless the relation between the potential conductance and EC cell design on one hand and potential and the pH on the other hand is well understood. At 1.20 V the background current, 80 nA, is about 10 and 5 times larger than those of the 0.80 V and 1.0 V for the 0.05 M $NaClO_4$ solution in water. Using a high impedance cell translates this high current into a larger IR potential drop along the electrode surface for 1.20 V for the same solution. As mentioned earlier the change in conductance results in IR drop variation and thus a continuous change in electrode potential along the surface during the course of the gradient. Despite the equal conductance shift for all three potentials, it has a higher impact in terms of the change in IR drop at 1.20 V than for 0.80 V or 1.0. At 1.20 V more impurities undergo electrolysis than at 0.80 V and 1.0 V, the electrode surface condition is different, and more importantly even the solvent (e.g., water) is being oxidized which is not the case for 0.80 V and probably 1.0 V. The continuous change of potential along the electrode surface varies the extent of oxidation more, causing larger baseline shifts. In addition pH gradient has greater impact on the residual current change at higher potentials where water decomposition and/or other pH dependent electrolysis reactions occur to a greater extent.

Table 6 shows the maximum change in residual current and the percentage of the change with respect to the initial value of background

Table 6. Residual current change for hydro-organic gradient at different potentials.

E (V)	Δi_r^{max} (na)[a]	% change[b]
0.80	6	75
1.0	14	78
1.20	202	269

---

[a] $\Delta i_r^{max} = i_r^{max} - i_r^{initial}$.

$\Delta i_r^{initial}$ = residual current corresponds to solvent A.

[b] $\% \text{ change} = \frac{\Delta i_r^{max}}{i_r^{initial}} \times 100.$

Table 7. Hydro-organic step gradient.[a]

Gradient % CH_3OH Change		i_{r_1} (nA)[b]	Δi_r (nA)[c]	$k_1 \left(\frac{\mu mho}{cm}\right)$[b]	$\Delta k \left(\frac{\mu mho}{cm}\right)$
0	20	20	+8	4860	-1170
20	50	28	+5	3690	-870
50	70	33	+2	2820	-60
70	100[d]	35	+2.5	2760	+837

[a] E = 1.0 vs Ag/AgCl, other conditions as Figure 12b.

[b] i_{r_1}, k_1 = residual current and specific conductivity corresponding to the initial mobile phase composition in step gradients.

[c] $\Delta i_r = i_r^{final} - i_r^{initial}$.

[d] Passes through a maximum at ~80% CH_3OH, corresponding i_r ~40 nA.

current. It should be noted that as a measure of gradient compatability Δi_r^{max} is the important factor and not the percent change. For example while the Δi_r^{max} value at 1.00 V is more than twice that of the 0.80 V, their corresponding percent changes are almost equal. For practical purposes a residual current change of 6 nA is more "acceptable" than 14 nA, no matter what the percentage of change. What matters, however, is the operating sensitivity which determines whether a Δi_r is acceptable. For example at 50 nA full scale, a 6 nA change at 0.80 V and even 14 nA for 1.0 V are considered tolerable, and at 500 nA full scale the baseline shifts are barely noticeable at these potentials. On the other hand at the sensitivity of 5 nA full scale, none of the corresponding shifts at three potentials are useful. Worst of all a Δi_r of 202 nA at 1.20 V is totally unacceptable for any typical sensitivity range used in EC detectors. Generally a sensitivity of 50 nA full scale is sufficient for the detection of most electroactive compounds at ng levels.

By operating at low potentials and narrowing the gradient range, one can use hydro-organic gradients with EC detectors successfully. The work reported by St. Claire et al.[67] is probably a good example of the potential use of gradient elution with EC detectors. The initial mobile phase composition of 10% methanol in a pH 5.0 buffer was changed to $CH_3OH:THF:H_2O$ (75:5:20) in 10 min, i.e., a gradient range of 70% organic modifier. The derivatives of 21 amino acids (167 pmol each) were separated and detected at a working potential of 0.70 V vs Ag/AgCl and at a sensitivity of 500 nA full scale. Despite the fact that the final mobile phase composition has different conductance and pH from the initial solvent, the gradient induced baseline shift is almost nonexistant. Note that a prototype cell was used with the working, reference and auxiliary electrode placed in the vicinity of the thin layer region. No details were given about the cell, but apparently it is a low impedance cell in contrast to the one used in this work. If so, the change in conductance should have minimal or no effect on the baseline shift. In addition operating at potentials such as 0.70V or 0.80 V and a sensitivity of 500 nA full scale causes a small shift regardless of the cell design and organic modifier concentration range, recalling the data shown in Table 6 as further evidence.

The similarity between the variation of conductivity and residual current as a function of methanol concentration, shown in Figures 11 and 12, a-c, reveals the dependence of sloping baseline on conductance variation, especially at 1.20 V, bearing in mind that pH contribution is significant since the pH was not and could not be adjusted for the whole range. At higher potentials such as 1.20 V, buffering the solution and balancing the conductance over the gradient range is of critical importance in order to obtain an "acceptable" baseline shift. However, this cannot be achieved for a wide range of concentrations of water-methanol mixtures. The aqueous buffer systems are not totally operative in hydro-organic mixtures. In addition, as shown in Figure 11, the specific conductivity passes through a minimum, corresponding to maximum viscosity making it almost impossible to balance the conductance of any two hydro-organic mixtures of desired composition. Nevertheless gradient elution can still be done at higher potentials by further narrowing of the gradient range to make adjusting the pH and conductivity feasible. To investigate the nature of the sloping baseline at different methanol concentration ranges, step gradients were run at two different potentials (Tables 7 and 8). The gradient ranges were chosen according to

Table 8. Hydro-organic step gradient.[a]

Gradient % CH_3OH Change		i_{r_1} (nA)	Δi_r (nA)	k_1 $\left(\frac{\mu mho}{cm}\right)$	Δk $\left(\frac{\mu mho}{cm}\right)$
0	20	82	134	4860	-1170
20	50	216	49	3690	-870
50	70	265	31	2820	-60
70	100[b]	296	-126	2760	+837

---

[a] E = 1.20 V vs Ag/AgCl; other conditions as Table 7.

[b] i_r passes through a maximum at ~80% methanol with i_r ~ 308 nA.

conductance variation at different methanol compositions at descending, constant and ascending slopes of the curve. Tables 7 and 8 are the step gradient of 1.0 V and 1.20 V corresponding to Figures 12, a and b, respectively, which represent baseline shifting over the whole range of CH_3OH concentration. As shown in Table 8, while a change of CH_3OH concentration by 20% created a baseline shift with the size of 134 nA in one case (0 to 20), it only resulted in 31 nA for another step gradient of 50 to 70%. The large difference in the size of the shift for an equal change in methanol concentration is probably because the conductance variation for the first gradient (1170 μmho/cm) is about 20 times larger than the second one (-60 μmho/cm). It should be stressed that the pH changes are not equal for all the step gradients which can contribute to different shifts. The change in double layer structure for the former case where the methanol is being introduced into the totally aqueous system probably is more pronounced than for the latter case where the methanol:water ratio is 1:1 for the initial composition. As another example while a gradient of 20% to 50% methanol yields a residual current change of 49 nA and a conductance change of -870 mho/cm, another 30% change in methanol concentration, this time from 70% to 100%, results in almost equal variation in conductance (+837 mho/cm) but shifts the baseline to a greater extent (-126 nA). An overall larger residual current for the second gradient (i_{r_1} = 296 nA) produces a larger IR drop, making the baseline more susceptible to change even for equal resistance variation. In addition the change in double layer structure once again may be more pronounced for the transfer from a hydro-organic to a totally non-aqueous media, i.e., 70% to 100% gradient.

For the first three step gradients in Tables 7 and 8, the increase in CH_3OH concentration causes the baseline to rise asymptotically to a new plateau. This is not the case, however, for the gradient of 70% to 100% methanol, where the baseline passes through a maximum before reaching steady state at final mobile phase composition. This residual current maxima probably corresponds to the conductance minima, despite the fact that the minimum conductivity of the water-methanol mixture occurs at about 60% methanol composition while the i_r maxima is somewhere beteen 70% and 80% CH_3OH. Note the difference in conductance between 60% and 80% methanol solution is only 40 mho. Thereby the slope of residual current changes in the same CH_3OH concentration range as the conductance does.

Table 9 shows the effect of the surface condition of the electrode on the size of residual current and baseline shift. First the electrode was polished and electrochemically treated by cyclic polarization of the electrode at +1.50 V (vs Ag/AgCl) and -1.20 V each for 5 min. The second experiment was performed 24 hr later under identical conditions except the electrode was not polished by was electrochemically pretreated at +1.50 V and -1.20 V each for 8 min. As a result the size of residual current, and consequently the baseline shift, is much larger for the second day. It should be noted that the data shown in Table 9 cannot be compared to those of Tables 7 and 8. This is because a new glassy carbon electrode was used for the former whose condition was superior compared to the "old" one. In addition in the latter study, an acetate buffer was used instead of aqueous solution of 0.05 M $NaClO_4$.

In summary hydro-organic gradient elution with EC detectors is feasible at "low" potentials, approximately 0.80 V vs Ag/AgCl or even 1.0 V

Table 9. Effect of glass carbon surface condition on the gradient induced baseline shift.[a]

Electrode's Condition (Pretreatment Procedure)	i_r^{buffer} (nA)	Δi_r^{max} (nA)	$i_r^{CH_3OH}$ (nA)
Polished; EC treated +1.50 V and -1.20 V for 5 min	61	380	143
EC treated +1.50 V and -1.20 V each for 8 min	130	580	203

* * * * * *

Solvent A

0.01 M Acetate buffer +

0.04 M $NaClO_4$

Solvent B

0.05 M $NaClO_4$ in CH_3OH

---

[a] E = 1.20 vs Ag/AgCl.

(depending on the sensitivity desired) where the value of the residual current is small. At extreme potentials such as 1.20 V or higher where background current is large, gradient elution can be performed provided controlling the conductance and the pH of the mobile phase is possible (e.g., narrow gradient range). Kissinger et al.[69] reported a successful gradient between 40% and 60% methanol (where conductance change is minimum) with parallel dual electrodes at potentials of 0.75 V and 1.15 V vs Ag/AgCl, with a sensitivity of 100 nA full scale. The pH and ionic strength of the eluents were held constant with the same molar concentration of buffer and electrolyte. In addition to the crucial role of the pH and conductance, one should realize that electrochemically pure mobile phase, temperature control, and especially "clean" electrode surface would affect the baseline stability to a great extent.

CHAPTER 5

GRADIENT ELUTION MICELLAR LIQUID CHROMATOGRAPHY WITH ELECTROCHEMICAL DETECTION

Introduction

Gradient elution has been recognized as the solution to what is known as the "general elution problem."[74] The problem occurs in the analysis of complex samples containing many compounds with a wide range in relative retention. In such cases one cannot find a single mobile phase composition at which all the compounds in the sample are separated in a reasonable period of time. Isocratic elution is ineffective in these situations because either the early eluting peaks are poorly resolved or the late eluting bands have long retention times, prolonging the analysis time. The late eluting peaks are also broadened and tailed severely causing poor detection sensitivity for these compounds. These problems can be overcome by changing the mobile phase composition in the direction of increasing strength during the course of separation. The technique is called gradient elution, in contrast to isocratic which means equal strength. As a result the later eluting peaks elute faster and are much narrower, while the early eluting bands remain well resolved. In RPLC the usual mobile phase is a mixture of water and a miscible, less polar organic solvent such as methanol, acetonitrile, etc. Thus in gradient elution with hydro-organic mobile phases, increasing the concentration of organic modifier during the chromatographic process increases the strength of the mobile phase and therefore shortens retention times. The major disadvantage of hydro-organic gradient elution is that the composition of the stationary phase changes during the course of the separation. During the gradient process a significant amount of organic modifier is extracted by the bonded stationary phase. For re-equilibration, the column should be washed copiously with the initial mobile phase composition. This process can be very long and time consuming to the point that for repetitive analysis long isocratic separations may be considered more beneficial in terms of overall analysis time. This is not the case, however, with micellar gradient elution.[75]

In micellar chromatography the strength of the mobile phase can be increased by increasing the surfactant concentration. As a result gradient elution can be simply performed by changing the surfactant concentration during the run. Dorsey and Landy[76] have reported that no column re-equilibration is necessary after a micellar gradient. They have shown that a step gradient back to the initial mobile phase composition and flushing the pre-injector delay volume would make the system ready for the second injection. Careful statistical evaluation of retention measurements of an early eluting solute following micellar gradients and a step back to initial conditions was taken as the proof. Failure to completely regenerate a column after a gradient will cause wide variability from run to run in the retention of early eluting peaks.[74] However, a reproducibility of 5% in the retention data did indeed show that no column re-equilibrium is involved. The rapid gradient capability of micellar chromatography stems from the fact that there is always a constant amount of free surfactant (equal to CMC) in micellar solutions, and any change in total surfactant concentration only changes the concentration of micelles.[34] Assuming that only free surfactant molecules and not micelles are adsorbed onto the bonded stationary phase, the conclusion can be drawn that the maximum concentration of surfactant on the stationary phase should be equal to CMC. Thus micellar concentration gradients can be performed without altering the composition of the stationary phase as long as the initial gradient concentration is above the CMC. This belief was further verified from the perspective of the stationary phase.

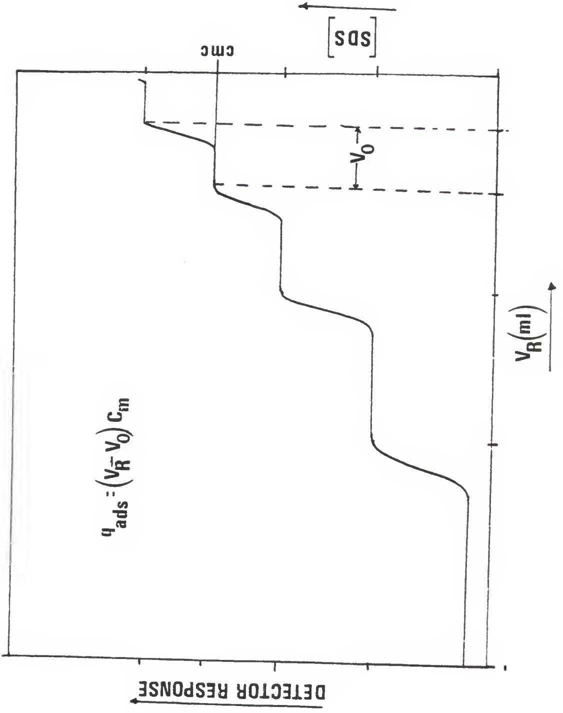
Adsorption Isotherm

Because of their heavy use as ion-pairing reagents, the adsorption of surfactants by reversed phase stationary phases has received much attention.[77-79] Adsorption isotherms were measured by determining the amount of surfactant adsorbed onto the stationary phase from frontal chromatography experiments (Figure 13). The breakthrough of the surfactant was monitored by both refractive index and conductivity detectors. Knox and Hartwick[77] have shown that it is extremely difficult to remove all adsorbed SDS from C_{18} phases, so a stepwise increase in concentration was used with each previous concentration being used as the new baseline. At each new concentration the column was first disconnected and the precolumn volumes flushed with the new concentration of surfactant. The break through volume (V_R) was measured to the top of the plateau, and the void volume of the column (V_0) was subtracted. The amount of surfactant adsorbed was then calculated as

$$q_{ads} = (V_R - V_0)C_m = AC_s \qquad (1)$$

where C_m is the concentration of surfactant in the mobile phase, C_s is the surface concentration of adsorbed surfactant, and A is the surface

Figure 13. Frontal chromatogram.


[SDS]
cmc
V_O
V_R(ml)
$q_{ads} = (V_R - V_O) C_m$
DETECTOR RESPONSE

area of the stationary phase. A was reported to be 235 m^2/g or 752 m^2 column.[80]

Figure 14 is the adsorption isotherm of SDS with a standard mobile phase of 397 n-propanol:water. The maximum concentration of surfactant adsorbed on the stationary phase occurs at a mobile phase concentration of approximately 10^{-2} M and gives a surface concentration of approximately 1.8 μmoles/m^2 of adsorbed SDS. Figure 15 is a log/log plot of surface concentration vs mobile phase concentration and again it is clear that there is apparent saturation of the stationary phase (vide infra). Figures 14 and 15, then, are supporting evidence for the conclusion that no column re-equilibration is necessary after a micelle concentration gradient. In fact these plots should break at the CMC value of the surfactant, as that represents the maximum concentration of free surfactant that will exist in solution. Because of the nature of the curvature of these plots, they are not true Langmuir isotherms, that is, they do not break when the stationary phase becomes truly saturated, rather, the break is a result of the micellization of the surfactant.

These isotherms differ significantly from those of Knox and Hartwick[77] who reported Freundlich type isotherms for SDS. While they reported mobile phase concentrations at or above the CMC value of SDS, it must be noted that their solvent was 20/80 methanol:water, and it is highly doubtful that micellization occurred even in their highest concentration solution. The CMC of SDS in pure water at 25°C is 8×10^{-3} M and has been reported to first decrease and then increase with small additions of methanol. In solutions of 0.27 mole fraction methanol, micellar aggregation of SDS molecules is precluded.[81] These facts both argue that micelles did not exist in their mobile phase. They measured the surface

Figure 14. Adsorption isotherm of SDS on a C-18 column.

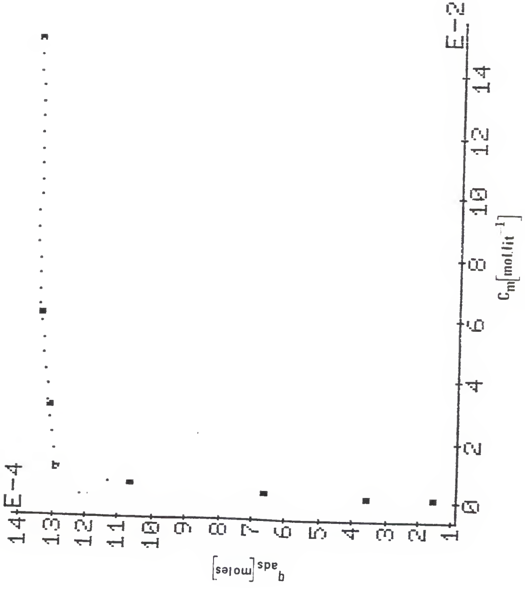
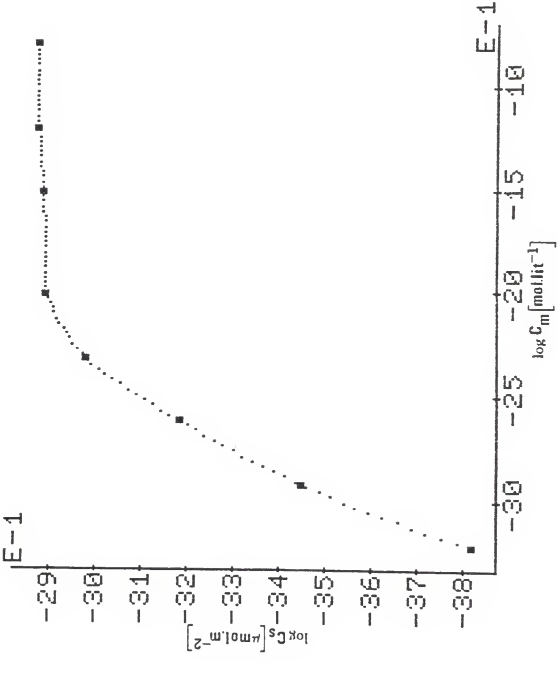

q_{ads} [moles]
E−4
1.4
1.3
1.2
1.1
1.0
9
8
7
6
5
4
3
2
1
0
2
4
6
8
10
12
14
E−2
C_m [mol lit^{-1}]

Figure 15. Log-log representation of Figre 14.


E-1
-29
-30
-31
-32
-33
-34
-35
-36
-37
-38
log C_s [μmol.m^-2]
E-1
-30
-25
-20
-15
-10
log C_m [mol.lit^-1]

concentration of SDS on ODS-Hypersil to be approximately 8 moles/m^2, a factor of four greater than that reported here.

The CMC value obtained from the adsorption isotherm (Figure 14) is 9.6×10^{-3} M. The conductometric method was also used for the determination of the CMC which is equal to 7.3×10^{-3} M. Figure 16 shows the specific conductivity of SDS solution as a function of the concentration. The slope of the conductance curve changes as micelle formation begins as the CMC. As a result the CMC value can be measured by extrapolating the two intersecting lines.[82] Dorsey et al.[42] used chromatographic method for the determination of CMC value by plotting log k' (capacity factor) as a function of log[SDS]. The CMC values for SDS measured by adsorption isotherm, conductometric and chromatographic methods are listed in Table 10. The solvent for all the methods was H_2O/1-propanol (97:3), and the temperature was 40°C for chromatographic method and 30°C for the other two methods. The hydrophobic interaction between the solute and the micelle in chromatographic method, which is non-existent for the other two methods, might be the cause of lower CMC value for the former. The CMC values are also temperature dependent, however, the overall variation is reported to be small.[36]

Gradient Elution: Micellar vs. Hydro-Organic Mobile Phases

The theory and advantages of gradient elution have been well documented.[74,83,84] There are some important distinctions, however, when considering reversed phase micellar chromatography as compared to traditional hydro-organic mobile phases.

Table 10. Comparison of CMC values for SDS measured by different methods.

Chromatographic Method[a]	Adsorption Isotherm[d]	Conductometric[d]
4.2 E -3 M[b]	9.6 E -3 M	7.3 E -3 M
5.3 E -3 M[c]		

* * * * * *

Solvent H_2O/1-propanol (97:3)

---

a Reference 42, T: 40°C.

b Solute: Nitrobenzene.

c Solute: Benzene.

d T: 30°C

Figure 16. Conductometric determination of CMC specific conductance vs surfactant concentration.

Figure 17. Cyclic voltammograms of 5×10^{-4} M hydroquinone in micellar solution at pH 2.50.

Scan rate: 25 mV/s

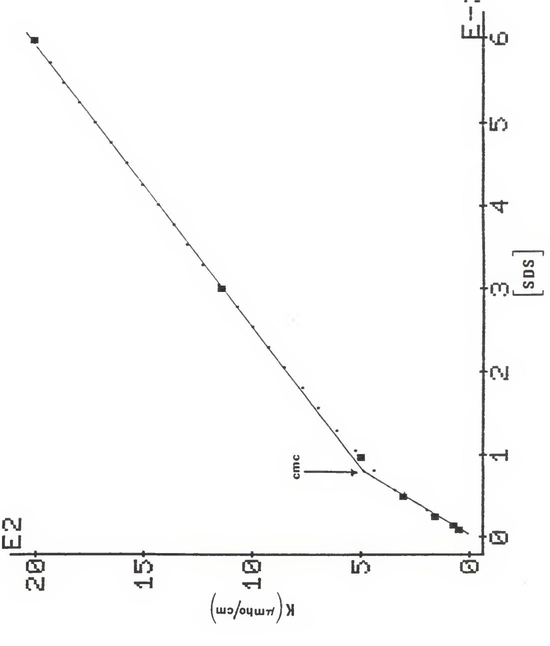
Solvent: 3:97 N-propanol:water

Supporting Electrolyte: 0.05 M $NaClO_4$

(a) 5×10^{-6} M SDS

(b) 5×10^{-4} M SDS

(c) 0.15 M SDS


E2
20
15
10
5
0
K (μmho/cm)
0
1
2
3
4
5
6
E-2
[SDS]
cmc

Hydro-organic mobile phases generally exhibit linear plots of log k' vs percent modifier (at least over the range of k' values of interest in gradient elution). This then dictates that for a linear solvent strength gradient, a linear gradient from solvent A to B is the preferred shape. However, with micellar mobile phases, linearity occurs when plotting log k' vs log[surfactant].[41,42] This then dictates that a convex gradient would generally be the preferred shape for micellar mobile phases. However, the difference in the quality of separation between a linear and convex gradient is often slight.[74]

A second difference in the two types of mobile phases is in selectivity. With hydro-organic mobile phases, plots of log k' vs percent organic modifier for different solutes are (approximately) linear, but often not parallel. This change in selectivity can lead to changes in band position with changing gradient steepness.[83] This change in selectivity is even more common with micellar mobile phases. Yarmchuk et al.[41] have shown that retention order reversals can occur with changes in surfactant concentration. This implies that elution order in an isocratic separation may be different than the elution order in a gradient separation.

Lastly a question that must be considered is that of the strength of micellar mobile phases. Grushka et al.[85] have calculated ° parameters of water-methanol mixtures as a function of temperature and mobile phase composition. The expression given by Snyder[86] for calculating ° in normal phase chromatography has also been used in RPLC using carbon adsorbents:[87]

$$\ln(k'_1/k'_2) = a(\varepsilon^\circ_2 - \varepsilon^\circ_1) \qquad (2)$$

where a is the molecular area of the solute and the subscripts 1 and 2 indicate solvents 1 and 2, respectively. By choosing one of the solvents as a reference state and setting $\varepsilon = 0$, equation 2 provides a method for comparing solvent strengths. Table 11 shows strength values at 40°C for both SDS and CTAB at various concentrations using benzene as a solute with both 100% methanol and 90:10 methanol-water as the reference solvent. For comparison, Grushka et al. reported a value of -0.0867 for 4:1 methanol-water with a reference of 100% methanol and decylbenzene as the solute. This indicates that 0.1 M SDS and CTAB are both considerably weaker reversed phase mobile phases than 4:1 methanol-water. This need not mean that micellar mobile phases must necessarily give longer separation times than hydro-organic mobile phases. As will be seen later, SDS concentrations as high as 0.3 M have been used successfully, and it is also possible to reduce analysis time through the use of shorter chain length stationary phases.

Electrochemistry in Micellar Media

Low concentrations of surfactants have been used in electrochemical analysis for many years, particularly as maximal suppressors in polarography. Only recently, however, has there been much interest in the utility of surfactant solutions above the CMC.[88-90] The lack of attention may be attributed, in part, to a poor understanding of the effects of surfactants on the double layer structure, as well as on the kinetics and thermodynamics of the electron transfer process. Electroanalytical techniques have been applied often to fundamental

Table 11. Strength of micellar mobile phases.

Reference State	Surfactant	Concentration	$\varepsilon°$
100% Methanol	SDS	1×10^{-2} M	-0.792
		5×10^{-2}	-0.679
		1×10^{-1}	-0.610
	CTAB	1×10^{-2}	-0.801
		5×10^{-2}	-0.664
90:10 Methanol-Water	SDS	1×10^{-2}	-0.681
		5×10^{-2}	-0.567
		1×10^{-1}	-0.498
	CTAB	1×10^{-2}	-0.690
		5×10^{-2}	-0.553

studies of micelles, but there is a paucity of information about electroanalysis in micellar solution.

Tables 12-16 show the cyclic voltammetry (CV) results characterized by parameters such as cathodic and anodic peak potential and the peak current ratio for hydroquinone in micellar solutions. The hydroquinone-quinone redox system is reported to behave irreversibly under many conditions at most solid electrodes.[23] Considering the relation

$$E_{pc} - E_{pa} = \frac{0.059}{n} \text{ V}$$

as a "useful diagnostic test of a Nernstian reaction,"[73] the hydroquinone-quinone redox system behaves irreversibly under the experimental conditions of Tables 12-16. Note that the difference between E_a and E_c can also be affected by uncompensated resistance of the solution. Although the scan rate used in this study was very slow (25 mV/s), the reversibility of the redox system should also be tested at other scan rates. The electrochemistry of species can differ dramatically in micellar media compared to that of homogeneous aqueous solution.[88-90] The solubilization of reactant, redox product, and especially intermediate radical species, which might lead to their stabilization, can all change the course of electrolysis of some compounds. It also can affect the kinetics of electron transfer and the value of peak potentials.[88-90] As shown in Table 12, the cathodic and anodic potentials as well as the kinetic parameters, shown by $E_a - E_{a/2}$ and $E_c - E_{c/2}$, change slightly as the surfactant concentration changes. The

Table 12. Cyclic voltammetry results for hydroquinone in micellar solution.[a] pH: 2.50; no supporting electrolyte added.

[SDS]	E_a (V)	$E_a-E_{a/2}$(V)	E_c (V)	$E_c-E_{c/2}$ (V)	E_c-E_a (V)	i_a (μA)	$\frac{i_a}{i_c}$
$5\text{x}10^{-3}$	0.44	0.06	0.19	-0.11	-0.25	8.4	1.22
0.05	0.41	0.05	0.25	-0.08	-0.16	10.1	1.16
0.10	0.41	0.05	0.25	-0.08	-0.16	10.0	1.14
0.15	0.40	0.04	0.26	-0.07	-0.14	10.1	1.10
0.20	0.40	0.04	0.26	-0.07	-0.14	10.4	1.14

---

[a] All potentials vs Ag/AgCl.

Scan rate 25 mV/s.

5×10^{-4} M Hydroquinone.

Solvent: H_2O:1-propanol (97:3).

Temperature: ambient.

E_a, E_c = anodic and cathodic peak potentials.

$E_{a/2}$, $E_{c/2}$ = anodic and cathodic half-peak potentials, i.e., the potential at the current equal to one-half of the peak current.

i_a, i_c = anodic and cathodic peak currents.

Table 13. Cyclic voltammetry results for hydroquinone in micellar solution.[a] pH: 2.50; 0.05 M $NaClO_4$.

[SDS]	E_a (V)	$E_a-E_{a/2}$ (V)	E_c (V)	$E_c-E_{c/2}$ (V)	E_c-E_a (V)	i_a (μA)	$\frac{i_a}{i_c}$
0.0	0.34	0.04	0.26	−0.05	0.08	10.4	1.06
$5x10^{-6}$	0.37	0.06	0.23	−0.06	0.14	10.6	1.10
$5x10^{-4}$	0.43	0.08	0.19	−0.09	0.24	7.53	1.12
$5x10^{-3}$	0.37	0.05	0.23	−0.07	0.14	8.12	1.14
0.05	0.36	0.05	0.24	−0.06	0.12	10.5	1.12
0.10	0.36	0.05	0.24	−0.06	0.12	10.4	1.11
0.15	0.34	0.03	0.26	−0.05	0.08	10.4	1.11
0.20	0.34	0.04	0.27	−0.04	0.07	10.4	1.06

---

[a] Other conditions same as in Table 12.

Table 14. Cyclic voltammetry results for hydroquinone in micellar solution.[a] pH: 5.50; 0.05 M $NaClO_4$.

[SDS]	E_a (V)	$E_a - E_{a/2}$ (V)	E_c (V)	$E_c - E_{c/2}$ (V)	$E_c - E_a$ (V)	i_a (μA)	$\frac{i_a}{i_c}$
0	0.33	0.08	0.18	-0.06	-0.15	8.55	1.04
$5x10^{-6}$	0.35	0.09	0.15	-0.10	-0.20	6.67	1.02
$5x10^{-4}$	0.40	0.11	0.09	-0.15	-0.31	6.67	1.12
$5x10^{-3}$	0.34	0.08	0.16	-0.10	-0.18	8.24	1.04
0.05	0.33	0.09	0.16	-0.09	-0.17	7.84	1.00
0.10	0.29	0.08	0.17	-0.08	-0.12	7.84	0.93
0.15	0.28	0.07	0.19	-0.06	-0.09	8.31	0.97
0.20	0.33	0.07	0.19	-0.06	-0.14	8.31	0.93

---

[a] Other conditions same as in Table 12.

Table 15. Cyclic voltammetry results for hydroquinone in micellar solution.[a] pH: 2.50; $[NaClO_4] + [SDS] = 0.25$ M.

[SDS]	E_a (V)	$E_a - E_{a/2}$ (V)	E_c (V)	$E_c - E_{c/2}$ (V)	$E_c - E_a$ (V)	i_a (μA)	$\frac{i_a}{i_c}$
0.0	0.35	0.04	0.25	−0.06	0.10	10.0	1.11
5×10^{-6}	0.38	0.06	0.23	−0.08	0.15	9.70	1.05
5×10^{-4}	0.47	0.09	0.14	−0.11	0.33	8.30	1.14
5×10^{-3}	0.38	0.06	0.23	−0.07	0.15	10.1	1.07
0.05	0.37	0.05	0.24	−0.06	0.13	10.1	1.05
0.10	0.37	0.05	0.25	−0.06	0.12	11.3	1.10
0.15	0.37	0.05	0.25	−0.06	0.12	9.80	1.07
0.20	0.36	0.05	0.25	−0.06	0.12	10.1	1.09

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[a] Other conditions same as in Table 12.

Table 16. Cyclic voltammetry results for hydroquinone in micellar solution.[a] pH: 2.50; 0.01 M SDS.

[SDS]	E_a (V)	$E_a - E_{a/2}$ (V)	E_c (V)	$E_c - E_{c/2}$ (V)	$E_c - E_a$ (V)	i_a (μA)	$\frac{i_a}{i_c}$
0.0	0.40	0.06	0.24	-0.07	0.16	9.76	1.13
0.05	0.38	0.05	0.23	-0.07	0.15	10.2	1.10
0.10	0.38	0.05	0.24	-0.06	0.14	10.5	1.12
0.15	0.37	0.05	0.23	-0.07	0.14	10.4	1.10
0.20	0.37	0.05	0.24	-0.06	0.13	10.1	1.05
0.25	0.36	0.05	0.24	-0.06	0.13	10.3	1.09

---

[a] Other conditions same as in Table 12.

different value obtained for 5 X 10^{-3} M SDS (Table 12) is mainly because of uncompensated resistance of the solution which shifts anodic and cathodic potentials in more positive and negative directions, respectively. The addition of 0.05 M $NaClO_4$ as suporting electrolyte (Table 13) provides the opportunity of studying electrochemistry of hydroquinone at very dilute surfactant concentration (e.g., 5 X 10^{-6} M) or in the absence of surfactant. Also the addition of 0.05 M $NaClO_4$ reduces the solution resistance which is the cause of potential shifts from the values reported in Table 12 to those in Table 13. Regardless of the results in 5 X 10^{-4} M SDS, E_a and E_c shift slightly (30 mV) to less positive and negative potentials as the SDS concentration is changed from 5 X 10^{-6} M to 0.15 and 0.20 M. As a result the peak potential difference (E_c - E_a) decreases by 70 mV in the same concentration range. The kinetics of the redox reaction also change slightly. As shown the parameters E_a - $E_{a/2}$ and E_c - $E_{c/2}$ decreased by about 20 mV indicating sharper anodic and cathodic peaks resulted as the SDS concentration was increased. In general the potential values approach that of the blank solution (no SDS) as the SDS concentration increases. Therefore one can conclude that these potential shifts are not caused by the reduction in the solution resistance simply because the results in the blank solution, the least conductive, is the same as the most conductive solution, i.e., 0.20 M SDS and 0.05 M $NaClO_4$. Interestingly at 5 X 10^{-4} M SDS the CV results of hydroquinone are different from those of the smaller and larger SDS concentration. The potential shifts are larger, the kinetics are slower and so the peaks are broadened (Figures 17 and 18). This behavior in 5 X 10^{-4} M SDS was also observed at other pH and $NaClO_4$

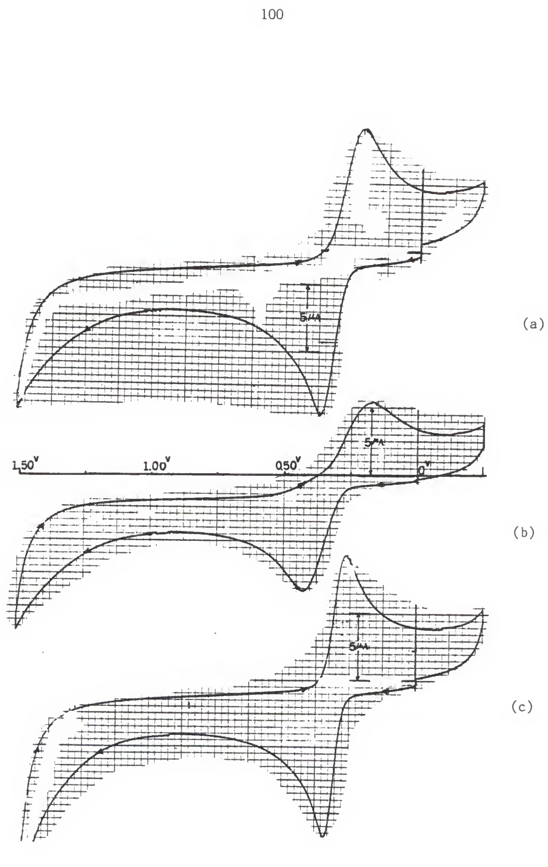
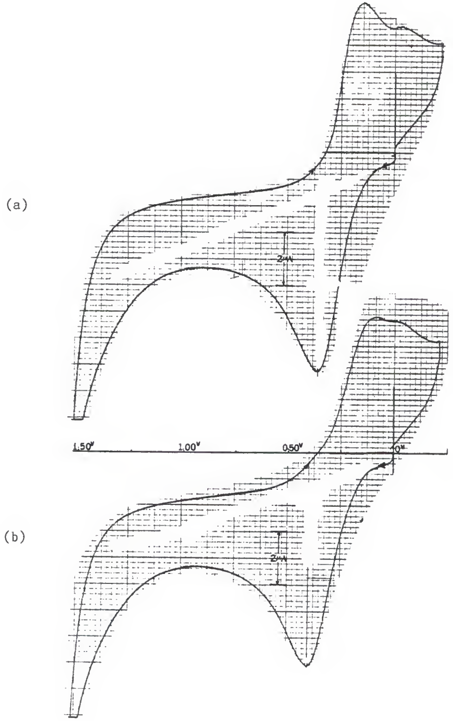
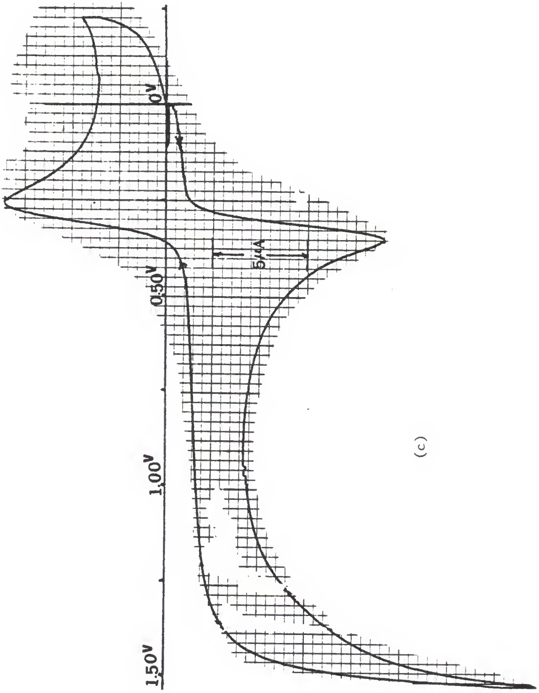

5μA
(a)
5μA
1.50V
1.00V
0.50V
0V
(b)
5μA
(c)

Figure 18. Cyclic voltammograms of 5×10^{-4} M hydroquinone in micellar solution at pH 5.50; other conditions as in Figure 17.

(a) 5×10^{-6} M SDS
(b) 5×10^{-4} M SDS
(c) 0.20 M SDS


(a)
2μA
1.50V
1.00V
0.50V
0V
(b)
2μA



(c)

concentrations (Tables 12-15). The peak currents ratio, i_a/i_c, is equal to 1.0 for a reversible reaction with stable products. Deviations from unity can be the result of "kinetics or other complications in the electrode process"[73] such as follow-up chemical reaction. As can be seen from Tables 12-16, the i_a/i_c values deviate from unity for almost all the cases. In addition to such possible kinetics or other complications in the electrode process, the one should also consider the error in peak current measurement due to uncertainty in choosing the baseline. At pH 5.50 (Table 14), the E_a values at all SDS concentration shift slightly to less anodic values as compared to those of the pH 2.50. The cathodic potentials, E_c, also more toward less positive values but to a larger extent. Thus the peak potential differences at pH 5.50 are larger than those of the pH 2.50. The anodic and cathodic peaks are more broadened at pH 5.50 compared to pH 2.50. However, the i_a/i_c values for the former are closer to unity than the latter. Once again the redox system seems to be more perturbed in 5×10^{-4} M SDS solution. As shown in Figure 18b, the cathodic peak is distorted compared to the well defined but broadened corresponding peak at pH 2.50 (Figure 17b). As the SDS concentration changed from 5×10^{-6} M to 0.15 M, anodic and cathodic peak potentials shift -70 mV (from 0.35 V to 0.28 V) and +40 mV, respectively. The anodic potential shift is larger at pH 5.50 than at pH 2.50. Surprisingly, however, as SDS concentration is changed from 0.15 M to 0.20 M, E_a shifts back toward more anodic (from 0.28 V to 0.33 V) with no change in cathodic potential. The potential results of 0.20 M SDS is again similar to that of the blank solution. As shown in Tables 15 and 16, the CV results in micellar solution with variable supporting electrolyte concentration are similar to the previous cases at pH 2.50.

To summarize the hydroquinone-quinone redox behavior in micellar solution is slightly different from that of in non-micellar media. However, more detailed investigation should be carried out before any general conclusion is drawn on the subject.

Compatibility of Micellar Gradient Elution with Electrochemical Detectors

The parameters involved in baseline shifts are the same for different mobile phases. However, micellar concentration gradients change the bulk properties of the mobile phase differently from those of an organic modifier concentration gradient in such a way that the control of the parameters of interest in EC detector baseline shift is easier for the former. This characteristic of micellar solutions, which can be considered as an advantage in gradient compatibility with EC detectos, stems from constant solvent composition during a micellar concentration gradient. The unchanging solvent composition makes the control of parameters such as pH, conductance and even mobile phase impurities easier compared to hydro-organic mixtures. Since water is the major constituent in the solvent (97% in this work), aqueous buffers can be used to balance the pH even in the presence of a small but fixed percentage of organic modifier (3% N-propanol). The conductance of a micellar solution is directly proportional to the concentration of ionic surfactant (Figure 16). Thus the conductance change during a micellar gradient can be greatly reduced, or even eliminated by using different supporting electrolyte concentration in the two surfactant solutions. For non-ionic surfactants the solution conductivity is totally controlled by the amount of added supporting electrolyte. Furthermore due to the constant concentration of free surfactant and fixed solvent composition, it is likely that the double layer structure remains virtually unchanged during the course of the gradient, reducing any possible contribution from this factor to the baseline shift. The feasibility of controlling both pH and conductance of micellar solutions provided the opportunity to study the effect of these factors on the baseline shift independently of each other at different working potentials. An anionic surfactant, SDS, was used throughout the experiments. The gradient concentration range was from 0.01 M, just above the CMC, to 0.40 M. This range is wider than necessary for many practical applications. However, the possibility of improving EC detector compatibility with micellar gradients over a wide concentration range and at high potentials was studied, knowing that a successful attempt under "extreme" conditions can be generalized for "normal" situations. Mobile phase impurities play an important role in the size of the residual current and so the baseline shift. Nevertheless neither the surfactant nor the supporting electrolyte salts were further purified. As mentioned earlier the solvent, water:N-propanol (97:3), was stored in a large container to ensure the homogeneity of electrochemical impurities for all experiments. No column was used, and all experiments were performed at ambient temperature. The tedious job of glassy carbon electrode pretreatment (both mechanical and electrochemical) was carried out before each experiment to improve the reproducibility of the surface condition of the electrode.

As shown in Tables 17 and 18, the applied potential has a similar effect on the baseline shift with both micellar gradients and hydro-organic gradients, i.e., at high potentials approximately 1.20 V, the size of the shift is much larger. The baseline, however, rises exponentially as a result of SDS gradient from 0.01 M to 0.40 M with and without $NaClO_4$ present. The only exception was observed for the gradient B (Table 18) at 0.80 V where the residual change is slightly negative. The magnitude of the residual current is larger for mobile phase solutions in gradient B compared to solutions in gradient A. This is due to the extra impurities from $NaClO_4$ in gradient B solutions. This larger residual current should logically cause a larger shift for equal changes in conductance and pH. But as can be seen, the conductance change, ΔK in gradient B is smaller than that of A, which results in a comparable Δi_r for both cases. Because no column was used, the induced frictional heat in the column is non-existent. The factors contributing to baseline shift are then ionic strength, pH, and changing impurity concentration. These factors as described in detail, have greater impact at high potentials. The effects of pH and ionic strength on the baseline shift were investigated independently at different potentials. Tables 19-21 show the effect of ionic strength in buffered media at 1.20 V, 1.00 V, and 0.80 V, respectively. The change in micellar concentration in gradient C, concentration gradient of micelle and supporting electrolyte ($NaClO_4$) in gradient D, and the variation of $NaClO_4$ concentration in gradient E are responsible for the change in conductance. As shown in Figure 16, the conductance of a micellar solution is directly proportional to the concentration of ionic surfactant, SDS in this case. The conductance behavior of SDS solutions is somewhat different from that of a 1:1 strong

Table 17. Effect of potential on baselin shift for micellar concentration gradient.[a]

E (V)[b]	i_{r_1} (nA)[c]	Δi_r (nA)[d]	k_1 $\left(\frac{\mu mho}{cm}\right)$[e]	Δk $\left(\frac{\mu mho}{cm}\right)$[f]
1.20	57	66	519	13,131
1.00	18	15		
0.80	8	4.0		

---

a Mobile phase gradient: A: 0.01 M SDS to 0.40 M SDS in 5 min.
F: 1 ml/min; ambient temperature.
Glassy Carbon electrode polished, pretreated at +1.50 V then −1.50 V, each for 2 min.

b E vs Ag/AgCl.

c i_{r_1} = residual current corresponding to initial mobile phase (0.01 M SDS).

d $\Delta i_r = i_r$ (at 0.40 M SDS) $- i_{r_1}$.

e k_1 = specific conductivity corresponding to initial mobile phase (0.01 M SDS).

f $\Delta k = k$ (at 0.40 M SDS) $- k$ (0.01 M SDS).

Table 18. Effect of potential on baseline shift for micellar concentration gradient.[a]

E (V)	i_{r_1} (nA)	Δi_r (nA)	k_1 $\left(\frac{\mu mho}{cm}\right)$	Δk $\left(\frac{\mu mho}{cm}\right)$
1.20	84	71	5070	12,210
1.00	29	14		
0.80	13	-2		

---

[a] Mobile Phase Gradient B: 0.01 M SDS and 0.05 M $NaClO_4$ to 0.40 M SDS and 0.05 M $NaClO_4$ in 5 min.

Other conditions same as in Table 17.

Table 19. Effect of ionic strength on baseline shift at constant pH: 2.35, E: 1.20 V vs Ag/AgCl.

	Mobile Phase Gradient			i_{r_1} (nA)	Δi_r (nA)	k_1 (µmho/cm)	Δk (µmho/cm)
C	0.01 M SDS 0.05 M $NaClO_4$	TO →	0.40 M SDS 0.05 M $NaClO_4$	60	16	7,620	12,900
D	0.01 M SDS 0.44 M $NaClO_4$	TO →	0.40 M SDS 0.05 M $NaClO_4$	98	-20	32,940	-12,540
E	0.01 M SDS 0.05 M $NaClO_4$	TO →	0.01 M SDS 0.44 M $NaClO_4$	67	30	7,860	25,080

Other conditions same as in Table 17.

Table 20. Effect of ionic strength on baseline shift at constant pH: 2.35, E: 1.0 V vs Ag/AgCl.

	Mobile Phase Gradient			i_{r_1} (nA)	Δi_r (nA)	k_1 (μmho/cm)	Δk (μmho/cm)
C	0.01 M SDS 0.05 M $NaClO_4$	$\xrightarrow{TO}$	0.40 M SDS 0.05 M $NaClO_4$	25	+7	7,620	12,900
D	0.01 M SDS 0.44 M $NaClO_4$	$\xrightarrow{TO}$	0.40 M SDS 0.05 M $NaClO_4$	58	−22	32,940	−12,540
E	0.01 M SDS 0.05 M $NaClO_4$	$\xrightarrow{TO}$	0.01 M SDS 0.44 M $NaClO_4$	33	+17	7,860	25,080

Other conditions same as in Table 17.

Table 21. Effect of ionic strength on baseline shift at constant pH: 2.35, E: 0.80 V vs Ag/AgCl.

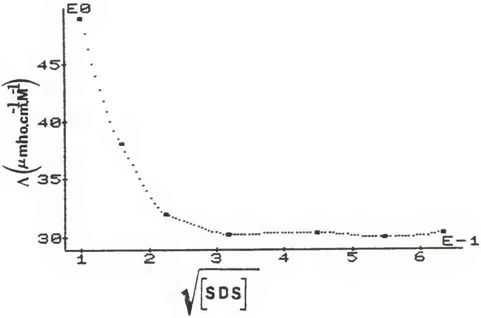
	Mobile Phase Gradient			i_{r_1} (nA)	Δi_r (nA)	k_1 (Δmho/cm)	Δk (μmho/cm)
C	0.01 M SDS 0.05 M $NaClO_4$	$\xrightarrow{\text{TO}}$	0.40 M SDS 0.05 M $NaClO_4$	18	-5	7,620	12,900
D	0.01 M SDS 0.44 M $NaClO_4$	$\xrightarrow{\text{TO}}$	0.40 M SDS 0.05 M $NaClO_4$	38	-23	32,940	-12,540
E	0.01 M SDS 0.05 M $NaClO_4$	$\xrightarrow{\text{TO}}$	0.01 M SDS 0.44 M $NaClO_4$	23	+16	7,860	25,080

Other conditions same as in Table 17.

electrolyte such as $NaClO_4$. This is shown in Figures 19 and 20 where the molar conductivity of SDS and $NaClO_4$ are plotted as a function of the square root of the concentration. In gradient C, which is the buffered form of gradient B, the residual current changes of 16 nA and 7 nA at 1.20 V and 1.00 V (Tables 19 and 20) are smaller than the corresponding shifts in the unbuffered media of gradient B, i.e., 71 nA and 14 nA (Table 18). Although a smaller shift is expected in buffered media (Chapter 4), one should realize that the decrease from 71 nA to 16 nA at 1.20 V and even 14 nA to 7 nA at 1.00 V is not solely because of controlling the pH. It is, however, partly due to the fact that in unbuffered media of gradient B, the pH is about 5 while in gradient C the solutions were buffered at a pH of 2.35. As mentioned earlier, at higher pH the residual current is larger and so is the baseline shift for an equal change in conductance. The role of the solution pH range can be seen by comparing the results of a unit pH gradient of 2.30 to 3.30 (Table 22, gradient F) and those of the pH gradient between 4.80 and 5.80 (Table 23, gradient G). As shown the size of the residual current shift is much higher for gradient G (pH = 4.80 - 5.80) at 1.20 V and 1.00 V, despite the smaller change in conductance (30 μmho/cm compared to 210 μmho/cm). Interestingly, however, in gradient F (Table 22), the Δi_r is only 4 nA, compared to 41 nA in gradient G, and shows the baseline shift is independent of pH changes at 1.00 V and low pH's, where the massive oxidation of water is non-existent. For pH gradient G at 1.20 V, the new baseline was achieved after70 min (instead of about 20 min) from the start of the gradient. For the first 20 min, the gradient induced baseline shift has an average rate of 3 nA/min, and from then on it drifts at a rate of 1 nA/min. The corresponding residual currents for

Figure 19. Molar conductance vs $\sqrt{[SDS]}$.

Figure 20. Molar conductance vs $\sqrt{[NaClO_4]}$.


E0
45
40
35
30
Λ(μmho.cm^{-1}.M^{-1})
E-1
1
2
3
4
5
6
$\sqrt{[SDS]}$

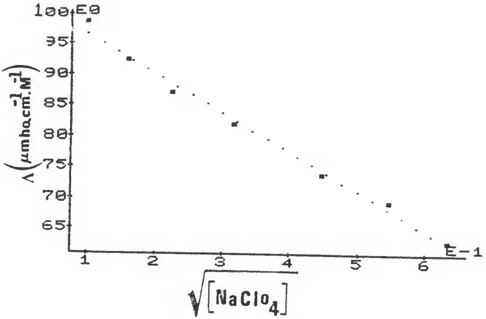

100
E0
95
90
85
80
75
70
65
Λ(μmho.cm^{-1}.M^{-1})
E-1
1
2
3
4
5
6
$\sqrt{[NaClO_4]}$

Table 22. pH gradient in micellar solutions at constant ionic strength.

Mobile Phase Gradient: F:

0.01 M SDS	$\xrightarrow{\text{TO}}$	0.01 M SDS
0.05 M $NaClO_4$		0.05 M $NaClO_4$
pH: 2.30[a]		pH: 3.30[a]

E (V)	i_{r_1} (nA)	Δi_r (nA)	Δk_1 (μ mho/cm)	Δk (μ mho/cm)
1.20	55	30	7,350	210
1.00	17	4		
0.80	10	1		

---

[a] Phosphate buffer.

Table 23. pH gradient in micellar solutions at constant ionic strength.

Mobile Phase Gradient: G:

0.01 M SDS	$\xrightarrow{TO}$	0.01 M SDS
0.05 M $NaClO_4$		0.05 M $NaClO_4$
pH: 4.80[a]		pH: 5.80[a]

E (V)	i_{r_1} (nA)	Δi_r (nA)	k_1 (μmho/cm)	Δk (μmho/cm)
1.20	155	75[b]	6,210	-30
1.00	76	41		
0.80	50	+4		

---

[a] Acetate buffer.

[b] After 30 min.

forward and reversed gradients are irreproducible. The adsorption of mobile phase impurities or the redox products on the electrode surface might be responsible for the drift and the irreproducible results. In general the time needed for EC detector to reach a new baseline upon a change in mobile phase composition (such as water to methanol or micellar concentration gradient) is about 5 to 15 min longer than that of the conductivity detector, despite the fact that the time constant of the former (0.5 sec) is half of the latter (1 sec). This time gap is partly due to the dead volume between the conductivity and EC detector (about 0.23 mL) and the volume between the working and auxiliary electrode (about 0.5 mL). Thus the current change due to a mobile phase gradient is seen on the recorder only after the volume between the two electrodes is totally swept with the corresponding mobile phase composition. However, a total dead volume of 0.70 mL cannot be a major contributor to approximately 10 min time gap with a flow rate of 1 mL/min. As mentioned earlier a solvent gradient changes the IR drop along the electrode surface, thus resulting in electrode potential variation. The time needed for the electrode to stabilize after the induced potential change is a major part of the time gap. In addition the extra time needed for the electrode to reach a steady state with the ever changing mobile phase composition should be considered. Interestingly the stabilization time of the electrode for a hydro-organic gradient is longer (5 to 10 min) than for a micellar gradient. the dramatic change of the solution electrode interface in a hydro-organic gradient compared to little or no change in the micellar gradient is likely responsible.

The gradient D shown in Tables 19-21 is a simultaneous micellar gradient and $NaClO_4$ reverse concentration gradient. As shown the micelle concentration increases from 0.01 M to 0.40 M, and the concentration of $NaClO_4$ decreases from 0.44 M to 0.05 M at the same time. The resultant conductance change is negative due to the decrease in [$NaClO_4$]. The factors causing the baseline shift are conductance change and the variation in the electroactive impurity concentration. The latter parameter plays an important role due to the addition of large amounts of $NaClO_4$ (0.44 M) to the 0.01 M micellar solution. As can be seen the residual current of 98 nA, 58 nA, and 38 nA for corresponding potentials of 1.20 V, 1.00 V, and 0.80 V are very large compared to other 0.01 M SDS solutions at pH 2.30. Surprisingly, however, the size of the baseline shift is equal to all three potentials for the gradient D. This was also observed for [$NaClO_4$] gradient in constant concentration of SDS (0.01 M) at potentials 1.00 V and 0.80 V (Tables 19-21). It should be noted that the Δi, of -23 nA and 16 nA at 0.80 V are surprisingly large for this potential. It is likely that the different electroactive impurity concentrations in $NaClO_4$ are the predominant factor rather than the conductance change. Electrode fouling is not likely, as all the forward gradients were followed by reverse gradients, were repeated at least twice, and the residual current values agreed within 5% for forward and reverse gradients.

As shown in Table 24, controlling the pH and the ionic strength results in small baseline shift even at high potentials such as 1.20 V (Δi = -8 nA). The conductance of the micellar solution was balanced by adding more $NaClO_4$ to lower SDS concentration (0.01 M SDS). As a result, the micellar gradient is once again accompanied by a $NaClO_4$ concentration gradient. Considering the small change in conductance, the negative baseline shift is due to the decrease in $NaClO_4$ concentration. The overall

Table 24. Micellar gradient at constant pH and ionic strength.

Mobile Phase Gradient: G:

0.01 M SDS	$\xrightarrow{\text{TO}}$	0.40 M SDS
0.226 M $NaClO_4$		0.05 M $NaClO_4$
pH: 2.35		pH: 2.35

E (V)	i_{r_1} (nA)	Δi_r (nA)	k_1 (μmho/cm)	Δk (μmho/cm)
1.20	86	-8	20,580	-60
1.00	30	-5	20,580	-60
0.80	21	-5	20,580	-60

results of different micellar mobile phase gradients at the three potentials are listed in Tables 25-27. At 1.20 V (Table 25), the effect of pH and ionic strength are clear as the baseline shift was reduced from 70 nA to only 8 nA by controlling these parameters. At 1.0 V (Table 26), these parameters can still influence the size of the disturbance. At 0.80 V (Table 27), however, pH does not play an important role (e.g., pH gradients F and G) simply because water is not being oxidized, and apparently the impurities whose oxidations are pH dependent and are electroactive at 0.80 V are not present. The conductance change is also unimportant at this potential as the size of the residual current is too small to create a considerable IR drop problem. The exceptions at 0.80 V are gradients D and E where the residual currents are unusually large due to the large concentration of $NaClO_4$ present. These exceptions have no practical significance as the use of concentrated solutions of supporting electrolyte is avoided in LCEC experiments.

In summary micellar gradient elution is compatible with EC detection. As mentioned earlier the "extreme" experimental conditions (such as wide gradient range, high working potential, large supporting electrolyte concentration, and no special purification of mobile phase components) result in the largest baseline shift. Under "normal" conditions where the concentration gradient range and the supporting electrolyte concentration gradient (to compensate for the conductance difference) are smaller, the baseline disturbance is even less than the reported values. Using electrochemically pure reagents and operating at lower potentials would further reduce the gradient induced baseline shift.

Table 25. Compilation of baseline shift results in micellar solution at E = 1.20.[a]

Mobile	Phase [SDS]	Gradient $[NaClO_4]$	Δi_r (nA)	$\Delta k \left(\frac{\mu mho}{cm}\right)$	pH
A	G[d]	-	66	13,131	U
B	G	0.05	71	12,210	U
C	G	0.05	16	12,900	2.35
D	G	G	-20	-12,540	2.35
E	0.01	G	30	25,080	2.35
F	0.01	0.05	30	210	G[b]
G	0.01	0.05	75	-30	G[c]
H	G	G	-8	-60	2.35

[a] Other conditions same as in Table 17.
U = unbuffered.
G = gradient.

[b] pH gradient 2.35 to 3.35.

[c] pH gradient 4.80 to 5.80.

[d] .01 M SDS to .40 M SDS.

Table 26. Compilation of baseline shift results in micellar solution at E = 1.0 V.[a]

Mobile	Phase [SDS]	Gradient [$NaClO_4$]	Δi_r (nA)	Δk $\left(\frac{\mu mho}{cm}\right)$	pH
A	G	-	15	13,131	U
B	G	0.05	14	12,210	U
C	G	0.05	7	12,900	2.35
D	G	G	-22	-12,540	2.35
E	0.01	G	17	25,080	2.35
F	0.01	0.05	4	210	G[b]
G	0.01	0.05	41	-30	G[c]
H	G	G	-5	-60	2.35

---

[a] Other conditions same as in Table 18.

U = unbuffered.

G = gradient.

[b] pH gradient 2.35 to 3.35.

[c] pH gradient 4.80 to 5.80.

Table 27. Compilation of baseline shift results in micellar solutions at E = 0.80 V.[a]

Mobile	Phase [SDS]	Gradient [$NaClO_4$]	Δi_r (nA)	Δk $\left(\frac{\mu mho}{cm}\right)$	pH
A	G	-	4	13,131	U
B	G	0.05	-2	12,210	U
C	G	0.05	-5	12,900	2.35
D	G	G	-23	-12,540	2.35
E	0.01	G	+16	25,080	2.35
F	0.01	0.05	1	210	G[b]
G	0.01	0.05	+4	-30	G[c]
H	G	G	-5	-60	2.35

---

[a] Other conditions same as in Table 25.

U = unbuffered.

G = gradient.

[b] pH gradient 2.35 to 3.35.

[c] pH gradient 4.80 to 5.80.

Analysis of Phenols by Micellar Liquid Chromatography with Electrochemical Detection

One of the known applications of LCEC is the analysis of phenols in environmental samples.[91-93] Due to their toxicity eleven phenols are listed as priority pollutants by the Environmental Protection Agency.

Figures 21-23 show the isocratic separation of 8 phenols at different SDS concentrations with EC detector at +1.20 V. The separation time of 96 min at 0.01 M SDS is reduced to less than 12 min by using a stronger mobile phase, 0.30 M SDS. The faster analysis time is achieved, however, at the expense of co-elution of two early eluting compounds, phenol and p-nitrophenol (peaks 4 and 5).

As mentioned earlier micellar mobile phases offer a different separation selectivity compared to that of hydro-organic mobile phases. This is clearly seen in the peak elution reversal of 6, 7 and 8 as the SDS concentration is changed from 0.01 M to 0.30 M. It should be mentioned that the SDS mobile phases were not buffered in the isocratic separations. Therefore the retention behavior of the compounds can be affected as the pH changes. However, the micellar concentration gradients shown in Figures 24-28, where all solutions are buffered, demonstrate the same selectivity. It is interesting to note that while the retention of late-eluting compounds such as p-chloro-, p-bromo- and o-nitrophenol is changed dramatically as a result of SDS concentration change, the retention of early eluting peaks like catechol, hydroquinone, and resorcinol remains unchanged under similar conditions. This is due to the varying hydrophobicity among these compounds. The hydrophobic compounds (peaks 6-8) partition extensively into the micelle, so their retentions are greatly affected by micelle concentration. The early eluting peaks partition to a much smaller extent, if any, thus their retentions are less influenced by micelle concentration. By choosing different initial and final micelle concentrations as well as different gradient steepness, the analysis time and selectivity can be greatly altered (Figures 24-28). For example the initial and final SDS concentrations of the gradient separations in Figures 24 and 25 are the same. However, a longer gradient time resulted in the separation of all eight compounds in 32 min (Figure 24) while a steeper gradient yielded the co-elution of peaks 6 and 7 and an analysis time of 28 min (Figure 25). Greater initial and final SDS concentrations and a shorter gradient time (steeper gradient) resulted in an analysis time of less than 16 min with the overlap of some peaks (Figures 26-28).

The other important feature which should be noticed in Figures 24-28 is the magnitude of gradient induced baseline shift which changes between 5 and 20 nA for different chromatograms. It should be mentioned that the electrode was pre-treated only once throughout all experiments, thus the change in electrode condition is mainly responsible for the large shift of approximately 20 nA. In fact the baseline shift observed under isocratic conditions of 0.20 M SDS (Figure 22) can only be explained by variation of the surface electrode condition.

There is no doubt that the separational selectivity and gradient compatibility of micellar mobile phases combined with excellent selectivity and low detection limits of EC detectors make the analysis of phenols in different complex mixtures more feasible.

Figure 21. Isocratic micellar chromatogram of phenols.

Mobile Phase: 0.01 M SDS, 0.05 M $NaClO_4$, 3:97 N-propanol:Water

Electrochemical Detection

E: 1/20 V vs Ag/AgCl

F: 1.0 mL/min

Peak Identification: (1) Hydroquinone, (2) Resorcinol, (3) Catechol, (4) Phenol, (5) P-nitrophenol, (6) O-nitrophenol, (7) P-chlorophenol (8) P-bromophenol

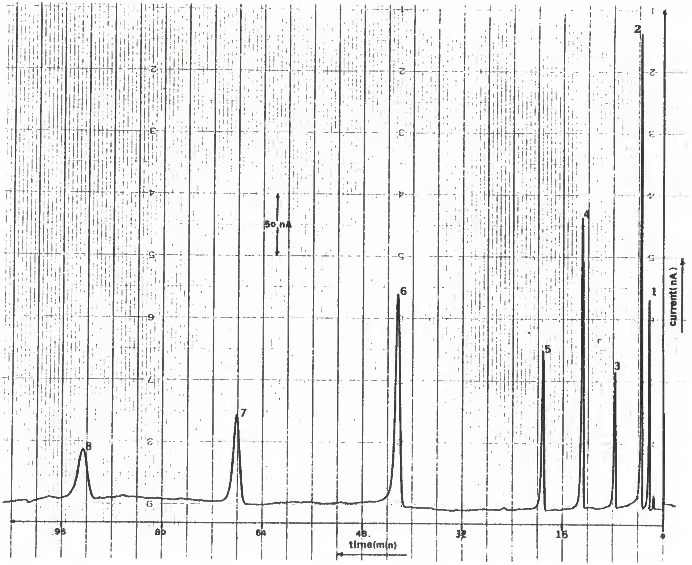

2
1
current(nA)
4
3
5
6
7
8
50 nA
96
80
64
48.
time(min)
32
16

Figure 22. Isocratic micellar chromatogram of phenols: 0.20 M SDS, other conditions same as Figure 21.

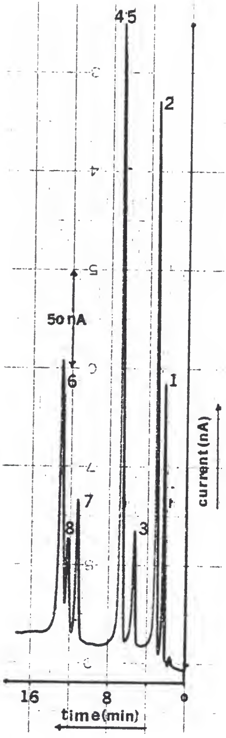

4 5
2
50 nA
6
1
current (nA)
7
3
8
16
8
0
time(min)

Figure 23. Isocratic micellar chromatogram of phenols: 0.30 M SDS, other conditions as in Figure 21.

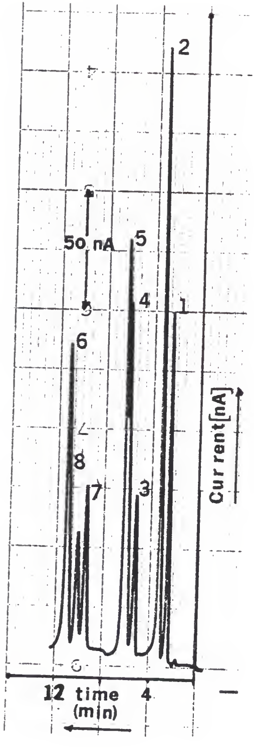

2
1
5
4
3
6
8
7
50 nA
Current [nA]
12 time 4
(min)

Figure 24. Micellar gradient chromatogram of phenols.

Electrochemical Detector at 1.20 V vs Ag/AgCl

Mobile Phase A: 0.05 M SDS, 3% n-Propanol, pH: 2.50 (phosphate buffer), $NaClO_4$ added to balance conductivity with solvent B

Mobile Phase B: 0.112 M SDS, 3% n-Propanol, pH: 2.50 (phosphate buffer)

Gradient Program 1: Solvent A for 8 min, A to B in 12 min

Flow Rate: 1 mL/min

Peak Identification: Same as Figure 21

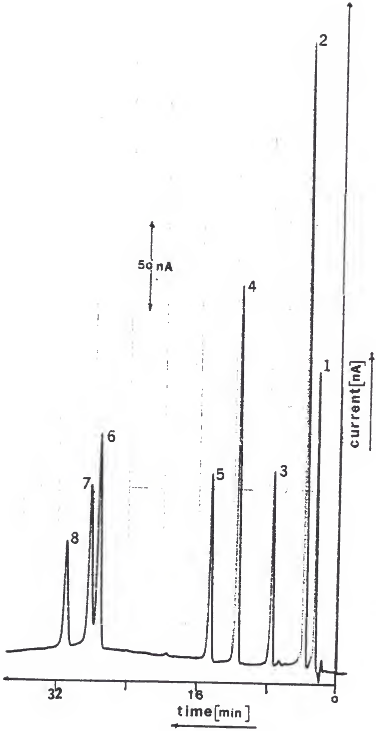
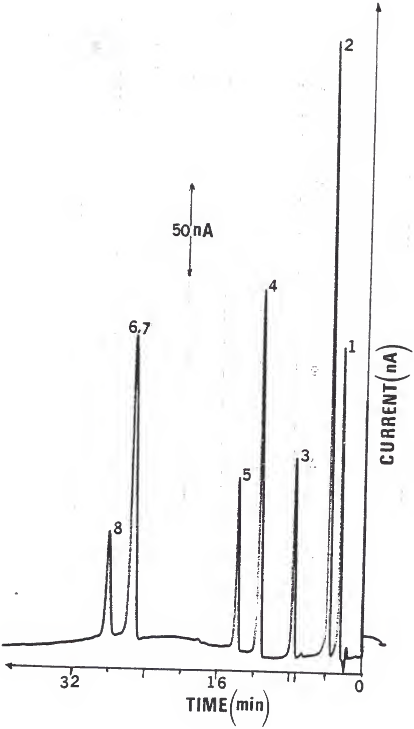

1
2
3
4
5
6
7
8
5o nA
current[nA]
32
16
o
time[min]

Figure 25. Micellar gradient chromatogram of phenols: conditions as in Figure 24.

Gradient Program 2: Solvent A to B in 15 min



2
1
3
4
5
6,7
8
50 nA
CURRENT (nA)
32
16
0
TIME (min)

Figure 26. Micellar gradient chromatogram of phenols.

Solvent A: 0.10 M SDS, 3% n-Propanol, pH: 2.50 (phosphate buffer), $NaClO_4$ added to balance conductivity with solvent B

Solvent B: 0.30 M SDS, 3% n-Propanol, pH: 2.50 (phosphate buffer)

Gradient Program 3: Solvent A to Bin 4min

Other conditions as in Figure 24

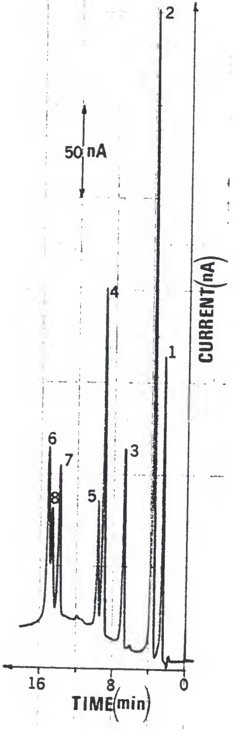

2
50 nA
4
1
CURRENT(nA)
6
7
3
8
5
16
8
0
TIME(min)

Figure 27. Micellar gradient chromatogram of phenols.

Solvent A: 0.112 M SDS, 3% n-Propanol, pH: 2.50 (phosphate buffer), $NaClO_4$ added to balance conductivity with solvent B

Solvent B: Same as Figure 26

Gradient Program 4: Solvent A to B in 10 min

Other conditions as in figure 24.

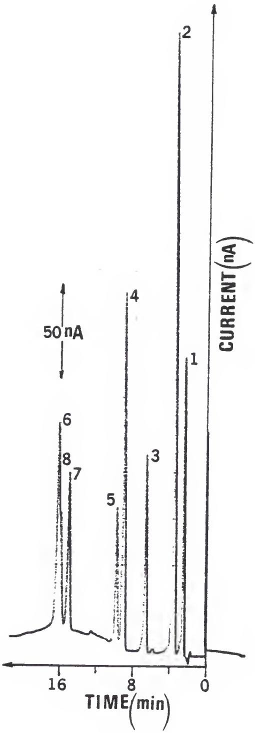

1
2
3
4
5
6
7
8
50 nA
CURRENT (nA)
16
8
0
TIME (min)

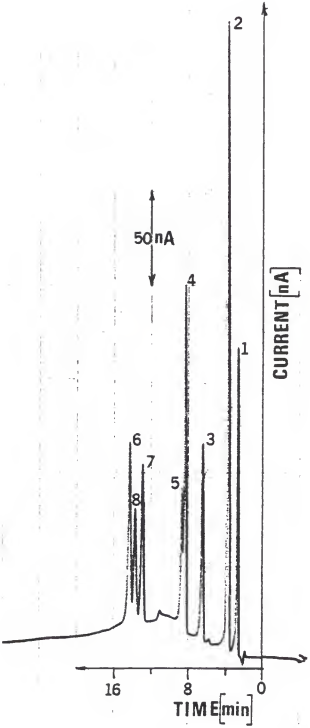
Figure 28. Micellar gradient chromatogram of pheonls.

Solvent A: 0.172 M SDS, 3% n-Propanol, pH: 2.50 (phosphate buffer), $NaClO_4$ added to balance conductivity with solvent B

Solvent B: Same as Figure 26

Gradient Program 5: Solvent A to B in 6 min

Other conditions as in Figure 24


2
50 nA
4
1
CURRENT [nA]
6
3
7
5
8
16
8
0
TIME [min]

CHAPTER 6

CONCLUSIONS AND SUGGESTIONS FOR FUTURE WORK

The feasibility of using amperometric electrochemical detection for the determination of polyaromatic hydrocarbons has been shown. With proper pretreatment of the electrode surface, the high operating potentials necessary for oxidation need not be prohibitive. As the number of rings increase, making LC determination the method of choice, the oxidation half-wave potential decreases, making electrochemical detection more attractive. This technique provides unique selectivities and can be especially useful when used in series with UV absorbance or fluorescence detection, or both.

There are contradictory reports in the literature on whether EC detectors are compatible with gradient elution. It is shown, however, that at low potentials and typical detector sensitivity, hydro-organic gradient elution can be used with EC detectors. At high potentials limiting the gradient range and controlling pH and ionic strength increases the applicability of gradient elution for EC detection.

Micellar concentration gradients are also shown to be compatible with electrochemical detection with minimal background current shifts. Gradient compatibility with EC detectors should now be added to the list of advantages of micellar liquid chromatography such as: rapid gradient capability, selectivity, low cost, low toxicity. Other more dramatic advantages are beginning to appear that should shift the role of micellar mobile phases from laboratory curiosity to practical utility.

The compatibility of micellar gradient elution with EC detector is demonstrated using an anionic surfactant throughout the study. This work should be continued with nonionic and cationic surfactants. The results are expected to be similar to those of the anionic surfactant. However, the adsorption of different surfactants on the electrode surface can have both a blocking effect and an electrostatic effect on the electrode process. This can accelerate or inhibit the charge transfer at the electrode surface which results in improved or limited sensitivity for EC detection. A study should be carried out to compare the effects of different surfactants on analytical figures of merit of EC detectors.

The analyses of aromatic amines and phenolic compounds in biological, pharmaceutical and environmental samples are the most common applications of LCEC. The rapid gradient capability, gradient compatibility with EC detection, and different separation selectivity as compared to hydro-organic mobile phases are compelling reasons to investigate the application of micellar LCEC for these groups of compounds.

Furthermore a better understanding of electrochemistry of these compounds in micellar solution is a precondition to the successful application of LCEC. Their redox behavior can be different in ordered systems compared to aqueous or hydro-organic media.

The cyclic voltammetry results for hydroquinone reported in this work should be considered a beginning step. More detailed experiments should be performed under different conditions, e.g., different scan rates, surfactant types, and concentrations, etc. In addition as shown in the chromatograms of phenols, hydroquinone does not interact with the micelles to a large extent. Thus the electrochemistry of (phenols) solutes with different hydrophobicity, and so different extent of interaction with micelles, should be studied.

One of the disadvantages of EC detectors is their small range of application, mainly due to the limited accessible potential range of the mobile phase. Micellar mobile phases may extend the range of compounds amenable to electrochemical detection. Franklin and Iwunze[94] have studied the oxidation voltammetry of organic compounds in micellar systems and found a number of organic compounds which gave well-defined anodic waves which had shown only slight shoulders or no wave in aqueous 2 M sodium hydroxide. Furthermore they found the anodic breakdown potential of water to be shifted by approximately +1 V in cationic micellar media. They concluded that the surfactant was oxidized at the electrode surface, forming a hydrophobic adsorbed film which excluded water but allowed oxidation of the organic solutes. Both of these findings should be studied for their applicability to chromatographic systems to broaden the scope of EC detector application.

Massive oxidation of water at high potentials (e.g., 1.20 V vs Ag/AgCl) produces large residual currents and is very much affected by pH changes, thus causing a large gradient induced baseline shift. Application of cationic surfactants whose oxidation results in the formation of a hydrophobic film and thus water exclusion from the electrode surface should also improve the compatibility of gradient elution with EC detectors.

Indirect photometric detection is a popular method of detection for UV transparent ions. A UV absorbing ion is added to the mobile phase producing a large background signal. The ion exchange between the UV transparent ion and the non-transparent ion then results in negative peaks. The possibility of indirect EC detection which extends the scope of EC detector applications should be investigated. Addition of electroactive compounds (or ions) to the mobile phase which form complex (or ion exchange) with non-electroactive compounds should yield negative peaks. In fact the same phenomena (negative peaks) were observed sometimes during the course of LCEC analysis of PAH's. The problem was solved by replacing the column. It is believed that the gradual corrosion of the stainless steel column body continuously introduced Fe^{2+} to the mobile phase which was being oxidized to Fe^{3+}, causing a large residual current. The injected PAH's apparently formed a complex with Fe^{2+}. Consequently upon the peak elution from the column, the Fe^{2+} concentration at the electrode surface was reduced resulting in a smaller residual current and negative peak.

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BIOGRAPHICAL SKETCH

Morteza Gholi Khaledi was born on March 16, 1956, in Shiraz, Iran. He attended Shiraz high school. He received a B. S. degree in chemistry from Shiraz University in 1978. He went directly to the University of Florida in January 1979, where he is working toward the Ph. D. degree in Chemistry.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

John G. Dorsey, Chairman
Assistant Professor of Chemistry

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

James D. Winefordner
Graduate Research Professor of
Chemistry

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Anna F. Brajter-Toth
Assistant Professor of Chemistry

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Christopher Riley
Assistant Professor of Pharmacy

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

David Richardson
Assistant professor of Chemistry

This dissertation was submitted to the Graduate Faculty of the Department of Chemistry in the College of Liberal Arts and Sciences and to the Graduate Council, and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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Dean for Graduate Studies and
Research